

Pasierbinska, Maria (2026) *Circulating microRNAs as diagnostic biomarkers in canine idiopathic and drug-resistant epilepsy.* MVM(R) thesis.

https://theses.gla.ac.uk/86358/

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This work cannot be reproduced or quoted extensively from without first obtaining permission from the author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Enlighten: Theses
https://theses.gla.ac.uk/
research-enlighten@glasgow.ac.uk

Circulating MicroRNAs as Diagnostic Biomarkers in Canine Idiopathic and Drug-Resistant Epilepsy

A Thesis Submitted by

Maria Pasierbinska DVM PhD MRCVS

Submitted in fulfilment of the requirements for the Degree of Master of Veterinary Medicine (MVM)

School of Biodiversity, One Health & Veterinary Medicine, University of Glasgow

May 2026

Copyright by Maria Pasierbinska 2026. All Rights Reserved.

Abstract

Background: Reliable and easily measurable biomarkers are needed to identify dogs with drug-resistant and idiopathic epilepsy. Circulating miRNAs may serve as both therapeutic targets and diagnostic or prognostic biomarkers for canine idiopathic epilepsy.

Hypothesis/Objectives: To determine whether specific circulating miRNAs are differentially expressed in dogs with idiopathic epilepsy and to evaluate their ability to distinguish drug-resistant cases from responders.

Methods: Circulating miRNA profiles were assessed in plasma samples from dogs with idiopathic epilepsy (drug-naïve, treatment-responsive, and drug-resistant) and healthy controls using expression profiling techniques. Candidate miRNAs identified from the analysis were subsequently validated using quantitative real-time PCR (qRT-PCR).

Results: Sequencing of circulating serum miRNAs identified clear expression differences between clinical groups. When comparing healthy controls with drug-naïve epileptic dogs, 45 miRNAs were significantly differentially expressed ($p < 0.05$), including miR-381, miR-214, and miR-224, which showed robust upregulation (fold change > 1.5; $p < 0.01$). In the comparison between drug-resistant and treatment-responsive epileptic dogs, six miRNAs were significantly altered, with miR-223 demonstrating reduced expression ($p < 0.05$) and miR-210 increased expression ($p < 0.05$) in resistant animals. RT-qPCR validation confirmed these sequencing-based findings: miR-214 was consistently upregulated in epileptic dogs relative to healthy controls ($p < 0.05$), supporting its diagnostic potential. miR-223 expression was significantly lower in drug-resistant dogs compared with responders ($p < 0.05$), while miR-210 was markedly elevated in resistant animals ($p < 0.05$). Together, these results highlight a subset of circulating miRNAs—particularly miR-214, miR-223, and miR-210—that show reproducible, clinically relevant expression changes and may serve as biomarkers for both epilepsy diagnosis and treatment stratification in dogs.

Conclusions and Clinical Importance: Differentially expressed miRNAs were identified, and their specificity, sensitivity, and expression levels were used to identify the most promising biomarkers. The results of this study indicate a potential role of miRNAs in the underlying molecular mechanisms of idiopathic and drug-resistant epilepsy in dogs, highlighting their use as potential biomarkers and establishing the basis for further studies.

Table of contents

Table of contents 3
Acknowledgements 5
Authors declaration 6
Abbreviations 7
1 Introduction and aims 8
1.1 Idiopathic epilepsy 8
1.1.1 Pathophysiology of idiopathic epilepsy 9
1.1.2 Drug-resistant epilepsy 10
1.1.3 Intrinsic Disease Severity 12
1.1.4 Pharmacokinetic Alterations 13
1.1.5 Pharmacodynamic Changes (Mutations/Downregulation of ASM Targets) 14
1.1.6 Genetic and Epigenetic Factors 14
1.1.7 Neuroinflammatory Processes 15
1.1.8 Clinical Implications for Human and Veterinary Medicine 17
1.2 Biomarkers in epilepsy 19
1.2.1 Genetic and epigenetic biomarkers 21
1.2.2 Proteomic and inflammatory biomarkers 22
1.2.3 Metabolomic biomarkers 23
1.2.4 Neuroimaging biomarkers 24
1.2.5 Electrophysiological biomarkers 25
1.3. MicroRNAs 26
1.3.1 miRNAs in the pathophysiology of epilepsy 27
1.3.2 The role of miRNAs in epileptogenesis 28
1.3.3 miRNAs as potential biomarkers in epilepsy. 29
1.4 Aims of the thesis 33
2 Materials and methods 34
2.1 Study design and ethics approval 34
2.2 Dog recruitment 34
2.3 Sample collection 35
2.4 RNA Library construction and sequencing 35
2.5 Bioinformatics analysis 35
2.5 Validation of selected miRNA with RT-qPCR 36
3 Results 38
3.1 RNAseq screening of serum from dogs 38
3.2 RT-qPCR identification of microRNA biomarkers 40
4 Discussion 42
4.1 Diagnostic Signatures of Canine Idiopathic Epilepsy 42
4.1.1 cfa-miR-214: Neurodevelopmental and Inflammatory Modulator 42
4.1.2 cfa-miR-381: Neural Stem Cell Regulator and Anti-inflammatory Agent 43
4.1.3 cfa-miR-224: Apoptosis Regulator and Signalling Pathway Modulator 43

4.1.4 Clinical Implications 43
4.2 Molecular Biomarkers of Drug-Resistant Epilepsy 44
4.2.1 miR-223 and the Excitatory/Inhibitory Balance 44
4.2.2 miR-129 and miR-210 as Markers of Refractoriness 44
4.3 Comparison with Previous Canine Research 45
4.4 Clinical Implications and Future Research 45
4.5 Limitations 46
4.6 Future directions 46
5 Conclusion 47
References 48
Supplementary material 62

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Rodrigo Gutierrez Quintana, for his continuous guidance, support, and valuable insights throughout the development of this thesis. His expertise, encouragement, and constructive feedback were essential to the completion of this work.

I would also like to thank my collaborators, Matias Perez* and Paul Capewell*, for their assistance, collaboration, and helpful discussions during this project. Their contributions and support were greatly appreciated.

Finally, I am grateful to everyone who supported me throughout my studies and contributed, directly or indirectly, to the completion of this thesis.

*School of Biodiversity, One Health and Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

Authors declaration

I, Maria Pasierbinska, declare that this thesis is my original work, undertaken solely by me except where specific acknowledgements are provided. It has not been submitted, in whole or in part, for any other academic degree or professional qualification. Some figures were created by me using the AI-based illustration software *FigureLabs* (<https://figurelabs.ai/>). Any reproduced figures from published sources were used only with the appropriate copyright permissions. The English language—grammar, syntax, and sentence structure—was reviewed and refined using *Grammarly* (<https://www.grammarly.com/>).

Abbreviations

ASD	Anti-seizure drug
ASM	Anti-seizure medication
AUC	Area under the curve
BBB	Blood brain barrier
Cfa-miR	*Canis familiaris* microRNA
CS	Cluster seizures
CSF	Cerebrospinal fluid
DRE	Drug-resistant epilepsy
EEG	Electroencephalography
FC	Fold Change
GABA	Gamma-Aminobutyric Acid
HS	Hippocampal Sclerosis
ICP	Intracranial pressure
IE	Idiopathic epilepsy
IVETF	International Veterinary Epilepsy Task Force
fMRI	Functional Magnetic resonance imaging
MCT	Medium-Chain Triglyceride
miRNA	MicroRNA
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate
P-GP	P-glycoprotein
RISC	RNA-induced Silencing Complex
ROC	Receiver Operating Characteristic
RT-qPCR	Quantitative Reverse Transcription Polymerase Chain Reaction
SE	Status Epilepticus
TLE	Temporal Lobe Epilepsy

1 Introduction and aims

1.1 Idiopathic epilepsy

Idiopathic epilepsy (IE) is a common chronic neurological disorder in dogs, affecting an estimated 0.6–0.75% of the general population. Its prevalence varies significantly across breeds such as Border Collies, Belgian Shepherds, and Labrador Retrievers (Pilla et al. 2020; Santifort et al. 2022; Berendt et al. 2015). This condition is characterised by recurrent seizures without identifiable structural brain lesions or metabolic causes, which often suggest a genetic predisposition (Kinsey, Belanger, and Oberbauer 2024). To standardize its diagnosis, the International Veterinary Epilepsy Task Force employs a three-tier confidence level system, with Tier I requiring an age of onset between 6 months and 6 years, normal interictal exams, and unremarkable blood and urine tests; Tier II builds upon this by adding normal bile acid stimulation, brain MRI, and CSF analysis; and Tier III further confirming characteristic EEG abnormalities (Table 1), (Risio et al. 2015). However, despite these diagnostic criteria, the current methods often lack the sensitivity and specificity for early detection and differentiation of idiopathic epilepsy from symptomatic forms, thereby highlighting an unmet clinical need for non-invasive, molecular-based biomarkers (Brennan et al. 2020; Szydłowska et al. 2023). A significant subset of idiopathic epilepsy cases in canines become drug-resistant, failing to respond adequately to conventional antiepileptic medications like phenobarbital and potassium bromide (Phochantachinda et al. 2023). This resistance to treatment underscores the critical need for novel diagnostic and prognostic biomarkers to improve therapeutic outcomes and patient management.

Tier Level	Requirements & Procedures	Evidence for Diagnosis
Tier 1	Age of onset between 6 months and 6 years; normal physical and neurological examinations between seizures; unremarkable minimum database	The most common level used in general practice to exclude metabolic or toxic causes
Tier 2	All Tier I criteria plus normal fasting/postprandial bile acids; normal brain MRI (to rule out structural lesions); and normal cerebrospinal fluid analysis	Increases confidence that no structural brain pathology is present
Tier 3	All Tier I & II criteria plus the identification of characteristic seizure-related abnormalities on an Electroencephalogram	The highest level of diagnostic certainty currently available in veterinary medicine

Table 1. International Veterinary Epilepsy Task Force diagnostic confidence levels (Tiers I–III) for idiopathic epilepsy in dogs (Risio et al. 2015).

1.1.1 Pathophysiology of idiopathic epilepsy

The pathophysiology of idiopathic epilepsy is fundamentally rooted in a "dysregulation of neural excitability," although it remains unclear if this stems from an excess of excitation, a failure of inhibition, or a combination of both (Fenner and Hass 1992). This imbalance can arise from a multitude of factors, including ion channelopathies, neurotransmitter dysregulation, and alterations in neuronal network connectivity (Costanza et al. 2025; Sumadewi et al. 2023). The lack of clarity regarding the specific molecular underpinnings of this imbalance, particularly in cases of drug-resistant epilepsy, necessitates further investigation into novel biomarkers. Unlike secondary epilepsy, IE is characterised by the "absence of recognisable causative lesions" and is often considered to have an inherited or suspected genetic basis (Koestner 1992). While conventional imaging may not reveal structural defects in IE, the condition likely involves "heterogeneous underlying pathologies," including subtle structural changes or cytoarchitectural disorganisation that facilitate the spontaneous and excessive neuronal firing characteristic of the disorder (Milne 2018; Olszewska et al. 2020). On a network level, seizures involve the synchronised activity of "bilateral cortical, subcortical, and brainstem networks" (Olszewska et al. 2020). In both humans and dogs, these "epileptogenic networks" often involve temporal lobe structures, such as the hippocampus and amygdala, which serve as common sites for seizure generation (Olszewska et al. 2020).

While the precise mechanisms underlying canine idiopathic epilepsy are not fully elucidated, research suggests a complex interplay of genetic, epigenetic, and environmental factors contributing to neuronal hyperexcitability and epileptogenesis. These factors can lead to alterations in neuronal excitability, synaptic plasticity, and network synchronisation, ultimately lowering the seizure threshold (Enright et al. 2018). For instance, dysregulation of the gut-brain axis, influenced by the gut microbiome, has been hypothesised to impact epilepsy pathogenesis, offering a potential avenue for dietary interventions in addition to standard antiepileptic medications (Silvestrino et al. 2024). Furthermore, the genetic heterogeneity observed in canine idiopathic epilepsy across various breeds, including specific inheritance patterns in breeds like the Belgian Tervuren, further complicates the understanding of its pathophysiology and treatment responses (Mandigers et al. 2024; Oberbauer 2003). The heterogeneity in disease progression and treatment response further complicates management, as evidenced by studies demonstrating varied outcomes and therapeutic failures even within genetically similar cohorts (Weissl et al. 2012). Moreover, subtle trace element imbalances, such as altered mercury burdens, have been observed in some dogs diagnosed with idiopathic epilepsy, indicating that metabolic factors might contribute to seizure aetiology and suggesting a need to re-evaluate current diagnostic classifications in line with human epilepsy (Rosendahl et al. 2023). Furthermore, histopathological studies of dogs in status epilepticus have revealed a reduced density of glutamate receptors in the lateral nucleus of the thalamus, which may impair the regulation of extracellular glutamate and trigger neuronal hyperexcitability (Hülsmeyer et al. 2015). Although direct thalamic

histopathology in humans with status epilepticus is limited, several clinical and experimental studies demonstrate that prolonged seizures induce maladaptive glutamatergic receptor regulation — particularly increased synaptic insertion and altered trafficking of NMDA and AMPA receptors — promoting sustained neuronal hyperexcitability (Alshehri et al. 2025). Reduced density of key glutamate receptor subtypes can paradoxically drive thalamic hyperexcitability by disrupting presynaptic inhibitory feedback, weakening GABAergic interneuron activation, and allowing extracellular glutamate to accumulate and activate extrasynaptic NMDARs. This combination of impaired regulation and network disinhibition lowers firing thresholds and promotes a hyperexcitable state (McGrath et al. 2022). Neuronal excitability is often amplified by neuroinflammation; mediators like high-mobility group box protein 1 act as initiators of inflammation, while cytokines such as interleukin-1β (IL-1β) upregulate the production of P-glycoprotein at the blood–brain barrier (Liyis et al. 2022; Shlobin and Sander 2022). This cytokine-driven induction of efflux transporters increases the active extrusion of many conventional antiseizure medications from the endothelial cells back into the circulation, thereby reducing their effective intracerebral concentrations. By limiting CNS drug penetration in this way, inflammation-mediated transporter overexpression provides a mechanistic link between neuroinflammatory signalling and the therapeutic intractability frequently observed in drug-resistant cohorts (Charalambous et al. 2023).

Additionally, intracerebral microdialysis has revealed increased extracellular concentrations of the excitatory neurotransmitters glutamate and aspartate in the cerebral cortex of epileptic dogs (Hülsmeyer et al. 2015). These molecular signatures, often dysregulated by the inflammatory cascades and excitotoxic processes described above, can permeate the blood-brain barrier and increases the active extrusion of many antiseizure medications, thereby reducing their intracerebral concentrations. As a result, diminished drug penetration contributes to the therapeutic intractability often observed in drug-resistant cohorts.

1.1.2 Drug-resistant epilepsy

Drug-resistant epilepsy (DRE), also termed pharmacoresistant or refractory epilepsy, poses a significant clinical challenge in canine idiopathic epilepsy. Approximately 20–30% of affected dogs fail to achieve satisfactory seizure control despite adequate trials of at least two appropriately chosen, dosed, and tolerated antiseizure medications such as phenobarbital, potassium bromide, imepitoin, or levetiracetam (Estey et al. 2017). This definition aligns closely with the International League Against Epilepsy criteria for human DRE, though veterinary applications emphasise breed-specific genetic predispositions and practical dosing constraints unique to canine practice. The underlying mechanisms contributing to DRE are multifaceted, involving dysfunctions of the blood-brain barrier, neuroinflammation, oxidative stress, and overexpression of drug transporters, all of which contribute to diminished therapeutic efficacy (Díaz-Coránguez et al. 2024; Leandro et al. 2019). Specifically, increased expression and activity of multidrug transporters, such as P-

glycoprotein (P-GP)—an ATP-dependent efflux pump that restricts xenobiotic and drug accumulation within the CNS— at the blood-brain barrier endothelium limit the entry of anti-seizure drugs into the brain parenchyma, thus reducing their effective concentration at epileptic foci (Garg, Joshi, and Medhi 2020).

In dogs, refractory cases often manifest with high seizure density, frequent cluster seizures, and episodes of status epilepticus—all robust predictors of poor ASM response and increased mortality risk. Research indicates that dogs presenting with high seizure density and frequent cluster seizures are significantly less likely to achieve seizure freedom or adequate control with anti-seizure medications (Packer et al. 2014). Specifically, higher seizure density and frequency are directly associated with a decreased likelihood of clinical remission (Packer et al. 2014). Dogs experiencing cluster seizures (CS) have a reduced probability of achieving seizure freedom compared to those without such episodes, mirroring findings in human medicine where both CS and status epilepticus are recognised predictors of pharmacoresistance (Packer et al. 2014). Beyond medication resistance, the presence of CS and status epilepticus (SE) serves as a major risk factor for both spontaneous death and euthanasia (Cagnotti et al. 2020). These conditions are considered life-threatening neurological emergencies, with mortality rates for SE estimated at approximately 25% in certain canine populations (Cagnotti et al. 2020). Consequently, cluster seizures are associated with significantly shortened survival times, as the combined emotional and financial burden of managing frequent, severe seizure episodes often drives the decision for euthanasia (Fig. 1), (Cagnotti et al. 2020).

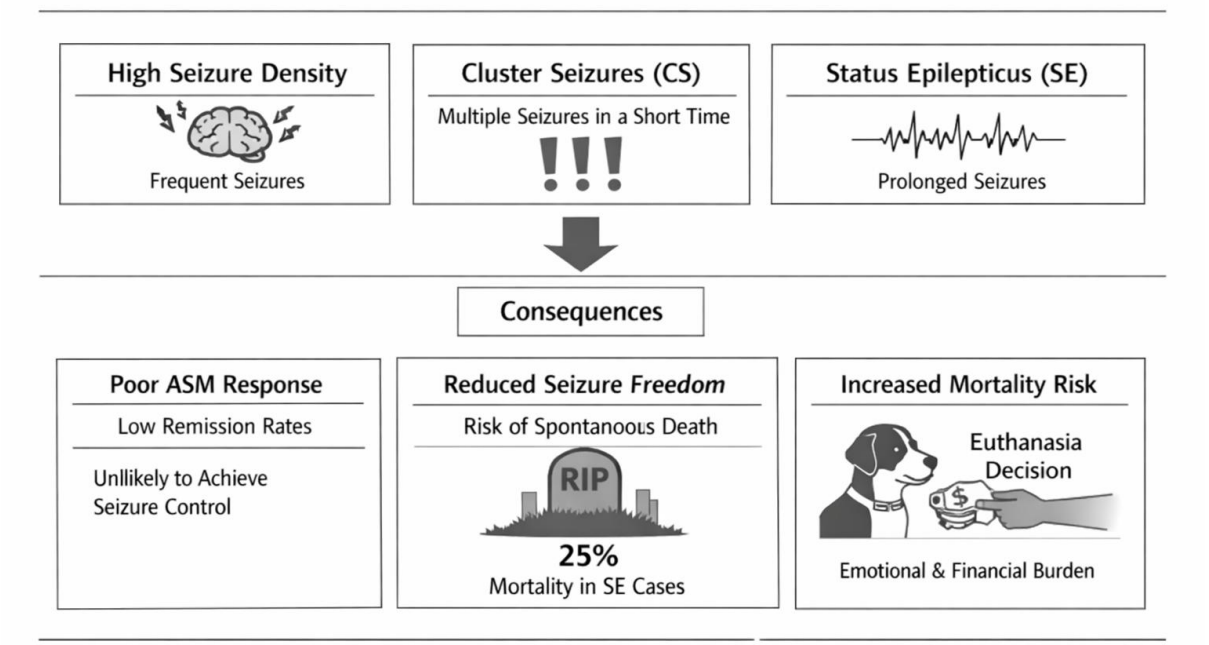


Figure 1: Schematic diagram illustrating the multifactorial nature of drug-resistant epilepsy in dogs (figure generated based on Cagnotti et al. 2020)

The pathophysiology of drug-resistant epilepsy is multifactorial and incompletely understood, involving interconnected mechanisms that contribute to antiseizure medication failure. Key hypotheses are described below (Fig.2).

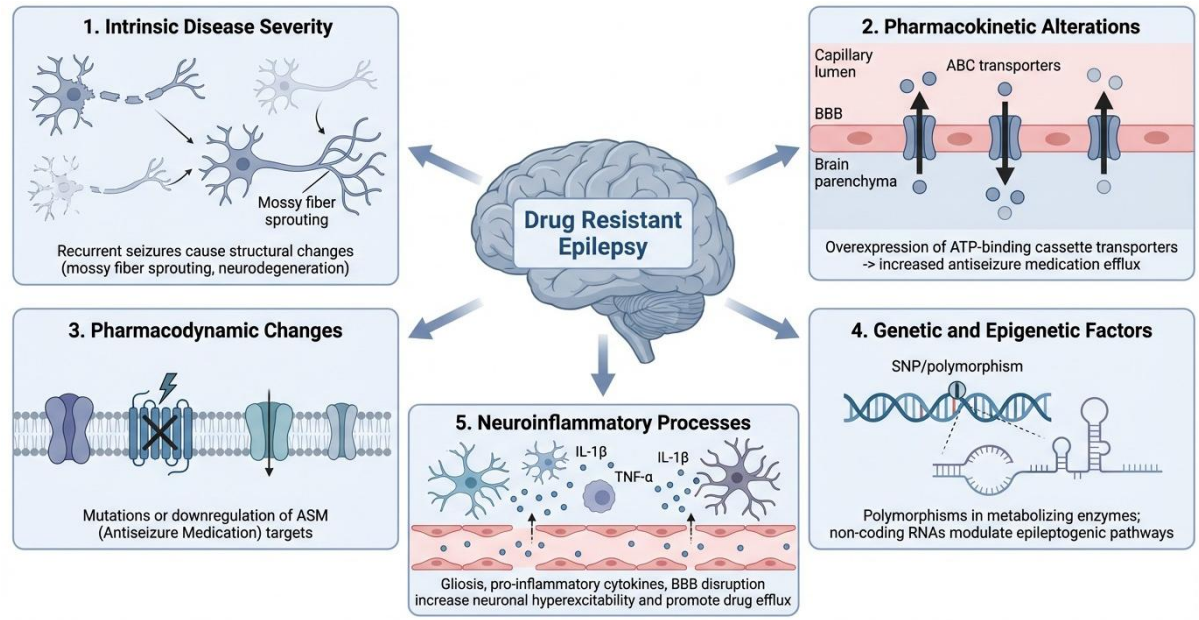


Figure 2. Major hypotheses in the pathophysiology of drug-resistant epilepsy.

1.1.3 Intrinsic Disease Severity

This concept posits that inherent neuropathological properties, such as progressive structural neuronal damage or baseline metabolic dysregulation, may create an environment intrinsically refractory to standard pharmacological modulation regardless of therapeutic optimisation (Potschka et al. 2023). Recurrent seizures drive epileptogenic network changes—such as mossy fibre sprouting, neuronal loss, and aberrant synaptic plasticity—rendering the epileptogenic focus less responsive to ASMs (Shlobin and Sander 2022). According to the neural network hypothesis, constant excitatory stimulation during seizures leads to neurodegeneration, synaptic reorganisation, necrosis, gliosis, and axonal sprouting, resulting in an abnormal network that reduces ASM efficacy (Shlobin and Sander 2022). Supporting molecular evidence shows abnormal expression of guidance and signalling molecules in the epileptic brain, promoting formation of new excitatory circuits through progressive sprouting in temporal lobe epilepsy (Tang, Hartz, and Bauer 2017). Cavarsan et al. provide a comprehensive review of mossy fibre sprouting (in humans and animal models) as a potential therapeutic target, particularly in mesial temporal lobe epilepsy with hippocampal sclerosis (Cavarsan et al. 2018). Complementing these ideas, the intrinsic severity hypothesis

theorises that drug-resistant epilepsy is an inherent property related to disease severity (Santana-Gomez, Engel, and Staba 2021); notably, Tang et al. highlight high pretreatment seizure frequency as an important predictor of refractory epilepsy, bolstering this view (Tang, Hartz, and Bauer 2017). However, this hypothesis does not fully account for cases where drug resistance develops over time or where patients with severe epilepsy achieve seizure control (Schmidt and Löscher 2005).

1.1.4 Pharmacokinetic Alterations

This perspective focuses on the capacity of individual patients to maintain therapeutic systemic drug concentrations, highlighting how inter-individual variations in hepatic metabolic enzyme activity or drug clearance rates can lead to sub-therapeutic plasma levels despite standardised dosing protocols. Overexpression of ATP-binding cassette transporters—particularly P-glycoprotein (P-GP)—at the blood-brain barrier actively effluxes anti-seizure medications (ASMs) from the central nervous system, reducing brain concentrations and driving treatment failure. This observation underpins the transporter hypothesis, a mechanism of drug resistance heavily conserved across species. In human medicine, this model is supported by evidence of *MDR1* mRNA overexpression in brain tissue resected from patients with refractory epilepsy (Tang, Hartz, and Bauer 2017). This exact pathophysiology is mirrored in veterinary medicine, where canine epilepsy models similarly demonstrate massive P-GP upregulation at the blood-brain barrier following prolonged seizure activity, leading to impaired ASM uptake and clinical pharmacoresistance (Pekcec et al. 2009; Potschka et al. 2023).Increased P-GP protein expression has been identified in epileptogenic tissue from human patients with refractory epilepsy and rodent models, correlating with poor brain penetration of multiple ASMs, including phenytoin, phenobarbital, felbamate, lamotrigine, topiramate, and levetiracetam (Hartz et al. 2017). ABC family transporters are similarly overexpressed in human drug-resistant brain tissue, restricting entry of lipophilic substrates and diminishing effective ASM concentrations at epileptic foci (Granata et al. 2009).

Genetic polymorphisms in transporter genes can further modulate their expression across species. For instance, extensively studied variations in the ABCB1 gene—such as the human C3435T single-nucleotide polymorphism or specific canine promoter and deletion mutations—are associated with altered P-GP levels and function (Chouchi et al. 2017; Mealey et al. 2023). Moreover, in both humans and dogs suffering from drug-resistant epilepsy, P-GP and other efflux transporters are upregulated in endothelial cells, astrocytes, and neurons (Leandro et al. 2019; Potschka et al. 2023). This upregulation can be induced by various inflammatory mediators, directly contributing to the diminished brain penetration of numerous antiepileptic drugs in both species (Löscher and Sills 2007; Rapacz 2018).

1.1.5 Pharmacodynamic Changes (Mutations/Downregulation of ASM Targets)

This hypothesis posits that resistance arises from genetic or acquired alterations in the molecular structures targeted by anti-seizure medications, such as specific ion channels or neurotransmitter receptors, which subsequently diminish the drug's therapeutic binding efficacy (Rawat et al. 2019). Pharmacodynamic changes encompass mutations or downregulation of ASM targets, including voltage-gated sodium channels, GABAA receptors, and synaptic vesicle proteins. Schmidt & Löscher describe the target hypothesis based on studies indicating reduced sensitivity of voltage-gated sodium channels to carbamazepine in epileptogenic brain tissue from AED-resistant patients (Schmidt and Löscher 2009). Furthermore, Shlobin & Sander report alterations in the beta subunits of voltage-gated Na^+ channels, resulting in protein misfolding or abnormal channel expression—featuring slowly inactivating channels that increase Na^+ currents and promote reduced drug sensitivity (Shlobin and Sander 2022). In addition, Abe et al. found that SCN1A and SCN2A (genes that encode the alpha subunits of neuronal voltage-gated sodium channels that are critical for electrical signalling in the brain)gene polymorphisms are associated with the pharmacodynamics of carbamazepine (Abe et al. 2008). Moreover, GABAA receptor subunit alterations were associated with resistance to phenobarbital in a rat model of temporal lobe epilepsy (TLE), and alterations in GABAA receptors likely explain resistance of status epilepticus to benzodiazepines (Schmidt and Löscher 2009). Shlobin & Sander discuss alterations in other receptors—including voltage-gated Ca^(2+) channels, GABAA receptors, and glutamate receptors—explored in cells and animal models (Shlobin and Sander 2022). Aicua-Rapun et al. report functional polymorphisms in the SCN1A gene associated with varying prescribed doses of phenytoin and carbamazepine (Aicua-Rapun, Andre, and Novy 2018).

1.1.6 Genetic and Epigenetic Factors

The “genetic hypothesis” suggests that inherited single-nucleotide polymorphisms may account for inter-individual differences in ASM susceptibility. Genetic and epigenetic factors encompass polymorphisms in drug-metabolising enzymes and non-coding RNAs that modulate epileptogenic pathways. Cytochrome P450 polymorphisms—such as those in CYP2D6, CYP2C9, CYP3A4, and CYP2C19—are involved in the metabolism of most AEDs, with low-activity alleles of CYP2C9 associated with decreased phenytoin clearance and increased toxicity (Singh et al. 2020). CYP450 isoenzymes, including CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP1B1, and CYP2E1, affect drug exposure in brain tissue (Silva-Alves et al. 2017). Studies of CYP2C9 and CYP2C19 genetic variations in drug-responsive versus drug-refractory epilepsy note that CYP450 genetic polymorphisms influence patient response to antiepileptic drugs through altered enzyme activity (Lakshmi et al. 2023). Beyond genetic variations in drug-metabolising enzymes, certain genetic polymorphisms—though not universally replicated across studies—have been linked to

antiepileptic drug resistance, representing a promising avenue for understanding pharmacoresistance (Álvarez, Serrano-Castro, and Serratosa 2007). Cárdenas-Rodríguez et al. highlight genetic variants in drug resistance-related genes, including voltage-dependent sodium and potassium channel genes, and metabolizer genes (Cardenas-Rodriguez et al. 2020). Finally, carrying CYP2C9 alleles with reduced activity causes a high risk of toxicity upon exposure to phenytoin (Aicua-Rapun, Andre, and Novy 2018).

1.1.7 Neuroinflammatory Processes

Neuroinflammatory processes, marked by gliosis, elevated pro-inflammatory cytokines, and blood-brain barrier disruption, heighten neuronal hyperexcitability and promote antiseizure medication efflux. In epilepsy, cytokine levels are notably elevated. For instance, Salem et al. report that various complement proteins are overexpressed in surgically excised tissue from epileptic patients, accompanied by increased serum concentrations of IL-1β, IL-1Ra, and IL-6 (Salem, Chetty, and Chetty 2023). Building on this, Zabrodskaya et al. describe how glial cells such as astrocytes and microglia produce and release cytokines and chemokines, with TNF-α, IL-1, and IL-8 typically signalling acute inflammation (Zabrodskaya et al. 2022). Similarly, Choi et al. found significantly higher IL-6 and MCP-1 levels in patients with a family history of epilepsy (Choi et al. 2009). Furthermore, neuroinflammation drives blood-brain barrier disruption, exacerbating pharmacoresistance. Shi et al. discuss how neuroinflammation—activated by immune components including reactivated glia, cytokines, and chemokines—can lead to neuronal network abnormalities that suppress endogenous antiepileptic systems (Shi et al. 2025). Complementing this view, Choi et al. demonstrate that pre-treatment with IL-1 receptor antagonists significantly reduced BBB disruption and delayed the onset of status epilepticus in seizure models, underscoring the role of cytokines and BBB damage in epilepsy (Choi et al. 2009). Shlobin & Sander further cite evidence that BBB dysfunction contributes to seizure pharmacoresistance (Shlobin and Sander 2022).

The above mechanisms frequently overlap, creating a complex interplay that determines individual responses to ASM therapy (Fig.3).

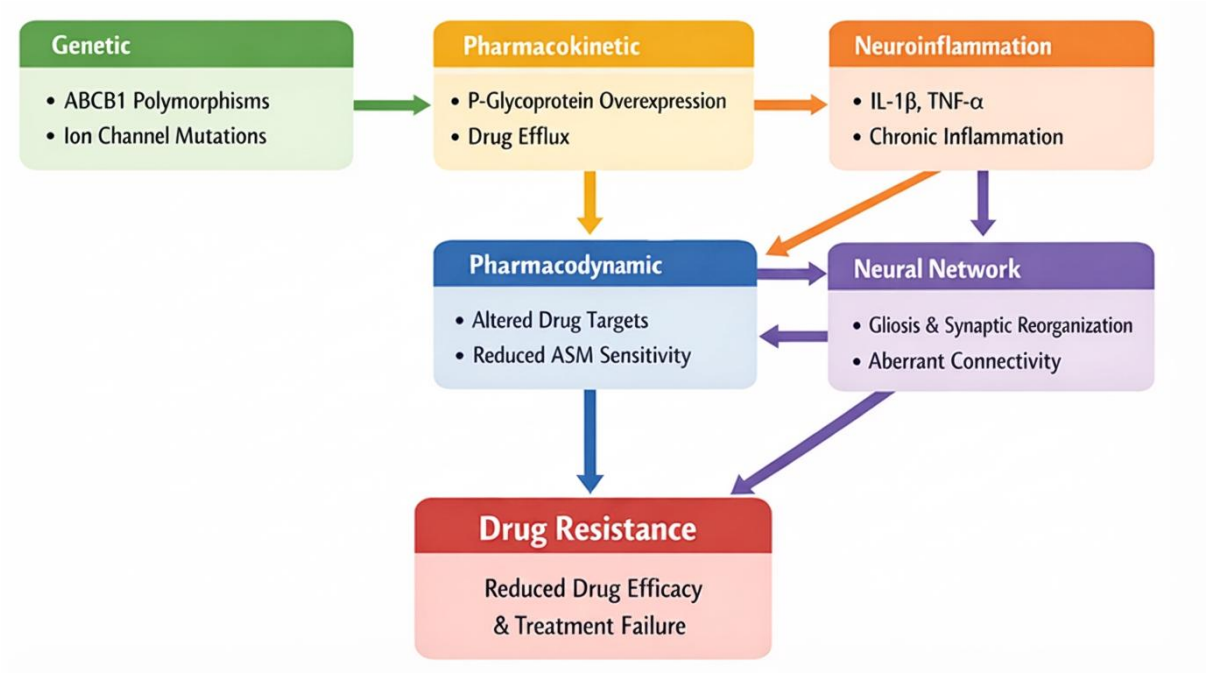


Figure 3. Key interconnections in the pathophysiology of epilepsy. Schematic representation of major mechanistic pathways contributing to epileptogenesis and drug resistance. Genetic mechanisms influence pharmacokinetic processes through ABCB1 polymorphisms, leading to P-glycoprotein overexpression and increased antiepileptic drug efflux. Neuroinflammatory pathways, mediated by cytokines such as IL-1β and TNF-α, upregulate ABC transporters and contribute to blood-brain barrier dysfunction, further altering pharmacokinetics. Genetic variants also affect pharmacodynamic mechanisms by modifying ion channel structure or function, reducing antiseizure medication sensitivity. Chronic neuroinflammation promotes gliosis, synaptic reorganisation, and aberrant neural network connectivity. All mechanisms converge to reduce drug efficacy and contribute to the development of drug-resistant epilepsy.

In canine idiopathic epilepsy, seizure severity—including frequency and intensity—strongly correlates with drug resistance, mirroring human findings and supporting the intrinsic severity hypothesis (Löscher 2022). Similarly, systemic or localised neuroinflammatory responses in canine patients may further modulate seizure thresholds by compromising blood–brain barrier integrity, thereby facilitating the emergence of hyperexcitable neural networks (Vezzani, Balosso, and Ravizza 2019; Bazhanova, Kozlov, and Litovchenko 2021).

The role of genetics in determining individual response to therapy is a key factor in veterinary neurology. Löscher notes that differences in breed distribution among ASM responders and non-responders indicate that genetics is a major factor in the medical intractability of canine epilepsy (Löscher 2022). Hülsmeyer et al. discuss the establishment of "breed-specific canine epilepsy phenotypes" and the ongoing genetic analysis of genes like ABCB1, which are known to influence drug resistance and metabolism in specific breeds (Hülsmeyer et al. 2015).

Estey et al. explain that while human epilepsy is often caused by mutations in ion channel genes (channelopathies), similar mechanisms are strongly suspected to exist in epileptic dogs, affecting how different breeds respond to sodium and calcium channel-targeting drugs (Estey et al. 2017). The "transporter hypothesis" involving the blood-brain barrier is a significant area of research in canine refractory epilepsy. Bankstahl & Bankstahl highlight that overexpression of efflux transporters like P-GP at the BBB is a primary mechanism of drug refractoriness in canine patients. This overexpression extrudes ASMs back into the blood, leading to sub-therapeutic drug levels at the site of the epileptic focus (Bankstahl & Bankstahl, 2017). Löscher confirms that these efflux pumps are regulated by drug-sensing nuclear receptors and can be overexpressed in the epileptic focus in ASM-resistant individuals, critically reducing functionally relevant drug levels (Löscher 2022). Fattorusso et al. add that neuroinflammation and BBB dysfunction are hallmarks of the epileptogenic zone in drug-resistant models, leading to P-GP induction in brain vessels and astrocytes (Fattorusso et al. 2021). Specifically, overexpression of efflux transporters like P-GP is implicated in reducing therapeutic ASM levels in the brain parenchyma (Löscher 2022; Santana-Gomez, Engel, and Staba 2021).

1.1.8 Clinical Implications for Human and Veterinary Medicine

Notably, differences between canine and human drug-resistant epilepsy (DRE) highlight translational opportunities and limitations. For instance, while genetic factors are significant in both species, breed-specific predispositions in dogs offer unique insights into specific genetic variants influencing pharmacoresistance (Löscher 2022). This comparative aspect allows for the development of targeted therapeutic strategies in veterinary medicine, which may also inform novel approaches for human patients (Nowakowska et al. 2022).

In humans, DRE predominantly arises in structural epilepsies (e.g., mesial TLE with hippocampal sclerosis, ~60% of cases). Resective epilepsy surgery is primarily indicated for patients with focal, drug-resistant epilepsy, which is most often associated with visible lesions such as hippocampal sclerosis, focal cortical dysplasia, tumours, or vascular malformations (Englot & Chang, 2014; Visocchi et al., 2017; Zhang & Kwan, 2019). Systematic reviews and meta-analyses report that overall seizure-freedom rates for resective surgery range from 55% to 70% (Zhang & Kwan, 2019). Specifically, focal cortical dysplasia and other lesional pathologies often fall within the 60–70% range for postoperative seizure control (Englot & Chang, 2014; Visocchi et al., 2017). Randomised controlled trials have demonstrated that surgery leads to significantly better outcomes than continued medical therapy, with roughly 58–77% of surgical patients achieving seizure freedom compared to only 7–8% of those on medical treatment alone (Arifin et al., 2021; Zhang & Kwan, 2019).

Conversely, canine idiopathic epilepsy and drug-resistant epilepsy are largely genetic or idiopathic in nature, mirroring human genetic generalised epilepsies. Specifically, canine idiopathic epilepsy is defined as a disease characterised by an enduring predisposition to seizures with a confirmed or suspected genetic background (Hülsmeyer et al. 2015; Płonek et al. 2022). With heritability estimates around 50%, the International Veterinary Epilepsy Task Force reports high hereditary grade (h^2) values in many breeds ranging from 0.33 to 0.47, highly suggestive of a strong genetic origin. Consequently, this genetic predisposition often results in diverse seizure semiologies and varying responses to antiepileptic medications across different breeds (Hülsmeyer et al. 2015). Furthermore, breed-specific variations can elevate heritability even higher. For example, estimates reach 0.77 in Belgian Tervuerens (Berendt et al., 2008) and up to 0.87 in Irish Wolfhounds (Casal et al., 2006). This high prevalence in specific breeds (up to 20% in some populations) further supports the role of breed-specific variants (Diness et al., 2013; Seppälä et al., 2012).

Dogs exhibit significantly faster metabolism for many anti-seizure medications compared to humans, often resulting in shorter half-lives that necessitate higher or more frequent dosing to maintain therapeutic levels (Charalambous et al., 2014; Löscher, 2022; Vaughan & Taylor, 1999). Unlike human medicine, where resective surgery for focal lesions is a common and effective option (Englot & Chang, 2014; Visocchi et al., 2017; Zhang & Kwan, 2019), canine patients often lack surgical alternatives (Hasegawa et al., 2022). This is largely due to the difficulty in accurately localising an epileptogenic focus in dogs and the frequently diffuse or multifocal nature of their epileptogenesis, which remains a major barrier to surgical intervention (Drobot et al., 2023; Hasegawa et al., 2021; Löscher, 2022).

In human drug-resistant epilepsy, clinical trials frequently emphasise the use of third-line ASMs such as lacosamide, brivaracetam, or cannabidiol (Dini et al., 2022; Fattorusso et al., 2021; Perucca, 2021). Conversely, canine clinical practice prioritises phenobarbital and imepitoin as first-line therapies (Charalambous et al., 2014; Gouveia et al., 2024; Löscher, 2022). Potassium bromide is a common add-on for refractory cases; while it is excreted renally, its lack of hepatic metabolism distinguishes its kinetics from many other standard ASMs (Gouveia et al., 2024; Lichtenauer et al., 2022; Vaughan & Taylor, 1999). Although the prevalence of DRE is comparable between the two species (~25–30%), canine patients experience cluster seizures and status epilepticus more frequently, with approximately 47% of dogs with idiopathic epilepsy experiencing at least one such episode, both of which correlate with a poorer long-term prognosis (Estey et al., 2017; Löscher, 2022; Roynard et al., 2021).

The understanding of drug resistance in epilepsy remains significantly hampered by an incomplete elucidation of its pathogenetic mechanisms (Yakimov et al. 2025). This complexity is underscored by the observation that numerous hypotheses, including pharmacokinetic, neural network, intrinsic severity, and

gene variant theories, each offer partial explanations, but none fully account for the diverse manifestations of pharmacoresistance (Tang, Hartz, and Bauer 2017).

Factors misclassified as resistance—such as subtherapeutic drug levels, pseudoresistance from concurrent illnesses, or initial misdiagnosis—account for a significant proportion of apparent refractory cases and often resolve with protocol optimisation, including therapeutic drug monitoring and client education (Tang, Hartz, and Bauer 2017). In confirmed DRE, ASM dose escalation or polypharmacy yields seizure reduction in some dogs but rarely achieves remission, with risks of adverse effects (Löscher 2022). Adjunctive options include ketogenic diets and MCT oil, which can cut seizures by ~50% in many refractory cases through ketone-driven GABAergic and anti-inflammatory effects. Cannabidiol shows meaningful responder rates, likely via GPR55 antagonism. Zonisamide and topiramate offer moderate benefit, while vagus nerve stimulation, responsive neurostimulation, microbiome-based approaches, and COX-2 (cyclooxygenase-2) inhibition target network excitability and inflammation (Nowakowska et al. 2022).Experimental approaches such as gene therapy targeting SCN8A or miRNA-based therapeutics to restore dysregulated profiles hold future promise but require validation in canine models (Henshall et al. 2016). This refractory subpopulation underscores the urgent need for precision medicine, including biomarker-driven stratification and deeper mechanistic insights to develop targeted therapies that address canine-specific pathophysiology (Agarwal et al. 2024).

1.2 Biomarkers in epilepsy

A biomarker is a measurable indicator of a biological state or condition, encompassing everything from molecular changes to physiological parameters (Ahmad, Imran, and Ahsan 2023). These indicators are crucial for diagnosing diseases, monitoring their progression, and evaluating the efficacy of therapeutic interventions (Romeo et al. 2021). In human neurology, biomarkers, including molecular and imaging markers, play a pivotal role in both clinical practice and research, aiding diagnosis, prognosis, and treatment stratification (Myrou et al. 2025). Specifically, in the context of idiopathic epilepsy, these biomarker types offer insights into underlying pathophysiology, helping to differentiate IE from structural causes, predict drug resistance, and monitor progression (Li et al. 2022; Mesraoua et al. 2023).

The identification and validation of robust biomarkers are critical for advancing precision medicine in epilepsy, in both human and veterinary medicine, particularly for facilitating early diagnosis, predicting treatment response, and monitoring disease progression. Despite their critical utility, however, established biomarkers for predicting seizure onset, treatment outcomes, or effective personalised therapeutic strategies remain largely unestablished in both human (Morita-Sherman et al. 2025) and canine epilepsy care (Potschka et al. 2023). In veterinary neurology, the identification of reliable biomarkers is particularly

critical for conditions like canine epilepsy, where definitive diagnoses and prognostic assessments can be challenging, thereby guiding more personalised treatment approaches and improving patient outcomes (Potschka et al. 2023).

Research into epilepsy biomarkers encompasses a wide array of molecular and physiological indicators, including neuroimaging, electroencephalography, and blood-based assays, all offering valuable insights into diagnostic and prognostic applications (Shah, Shah, and Parekh 2025). The identification of reliable biomarkers is considered a high-priority goal within the epilepsy community, as these tools could substantially enhance differential diagnosis, streamline pharmacotherapy selection, improve presurgical evaluation, and increase the cost-effectiveness of drug development and clinical trials (Engel 2011). Genetic and epigenetic biomarkers, for instance, identify heritable or regulatory variants that influence epileptogenesis. In human idiopathic epilepsy research, polymorphisms in ion channel genes and microRNA dysregulation serve as prognostic indicators (Henshall et al. 2016; Peplow and Martinez 2022; Sumadewi, Harkitasari, and Tjandra 2023). Similarly, in dogs, genetic markers identified through gene and exome sequencing aid in understanding epilepsy diagnosis and management and are also crucial for developing personalised treatment strategies (Cui et al. 2017). This approach is particularly critical given the high prevalence of drug resistance in canine idiopathic epilepsy, which necessitates precise diagnostic and prognostic tools to optimise therapeutic interventions (Kriechbaumer et al. 2022).

Beyond their general definition, epilepsy biomarkers are typically categorised into several distinct modalities, such as neuro-electrophysiological, neuroglial inflammation, and neuroimaging biomarkers, each offering unique insights into the complex pathophysiology of the condition (Fig.4), (Liu et al. 2024). Blood-based biomarkers, particularly circulating microRNAs, are emerging as non-invasive tools for diagnosing epilepsy and predicting treatment response, including drug resistance (Szydłowska et al. 2024).

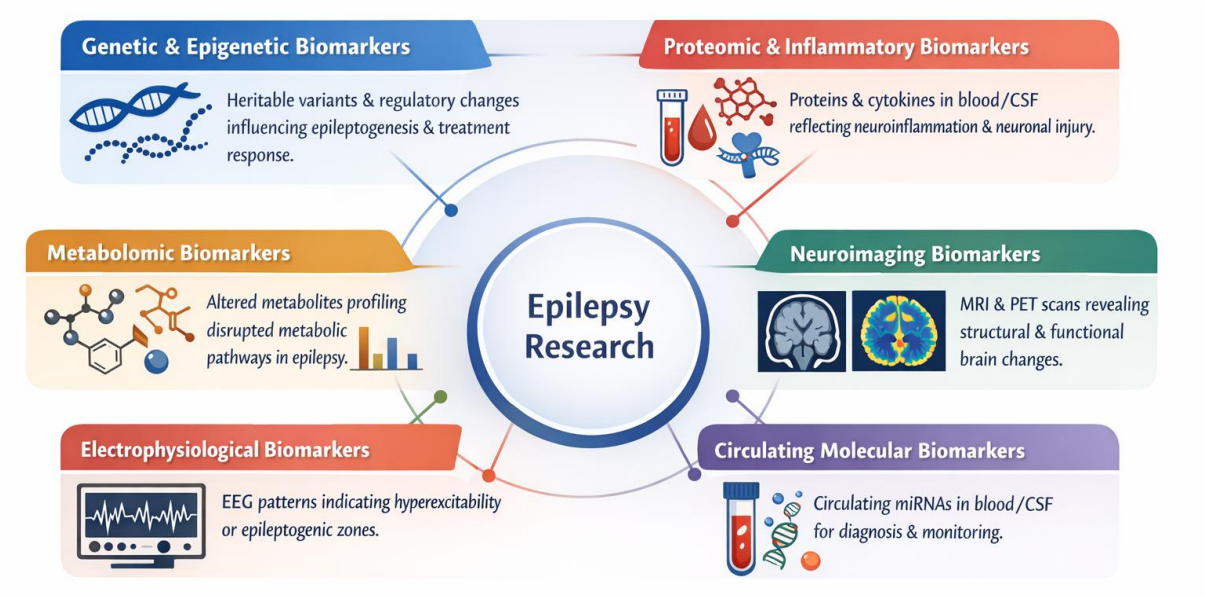


Figure 4. Types of biomarkers in epilepsy research. Overview of key biomarker categories relevant to epilepsy research, including genetic/epigenetic, proteomic/inflammatory, metabolomic, neuroimaging, electrophysiological, and circulating molecular biomarkers. *Icon generated using FigureLabs.*

1.2.1 Genetic and epigenetic biomarkers

Genetic and epigenetic biomarkers identify heritable or regulatory variants that influence epileptogenesis. These markers encompass alterations in DNA sequence, gene expression, and epigenetic modifications—such as DNA methylation and histone alterations—which modulate gene expression without changing the underlying DNA sequence, thereby influencing epilepsy susceptibility, phenotypic variability, and disease progression (Henshall and Kobow 2015). In human medicine, specific epigenetic modifications—including demethylation of the BDNF (brain-derived neurotrophic factor) promoter and increased histone H4 acetylation at the BDNF and glutamate receptor 2 loci—have been associated with seizure-induced pathological processes and heightened hippocampal excitability (Martins-Ferreira et al. 2022; Huang 2014). These epigenetic changes do not alter the DNA sequence itself; rather, they modify chromatin structure and thereby influence gene expression. In contrast, genetic variants in the 5-hydroxytryptamine (5-HT) receptor gene, sodium channel voltage-gated type I-alpha (SCN1A) gene, and gamma-aminobutyric acid (GABA) receptor gene represent alterations in the DNA sequence. Such variants can change the structure or function of the encoded proteins and are well-established indicators of epilepsy risk in humans (Xie et al. 2023).In idiopathic epilepsy research, polymorphisms in ion channel genes and microRNA dysregulation, such as altered levels of miR-4521 and miR-34c-5p, serve as prognostic indicators that help identify high-risk individuals and offer targets for antiepileptogenic interventions (Sumadewi, Harkitasari, and Tjandra 2023; Potschka et al. 2023).

Similarly, in veterinary medicine, genetic markers identified through gene and exome sequencing have become crucial tools for diagnosing and managing canine idiopathic epilepsy (Koskinen et al. 2017).

Notable examples include the LGI2 gene mutation, which is responsible for benign juvenile remitting epilepsy in Lagotto Romagnolo dogs, and the ADAM23 risk haplotype, which is significantly associated with adult-onset epilepsy across multiple breeds, including the Belgian Shepherd, Finnish Spitz, and Beagle (Seppälä et al. 2011; Koskinen et al. 2017). Interestingly, while ADAM23 is a common risk gene in dogs, related ADAM22 and ADAM23 variants are also associated with progressive encephalopathy in humans, highlighting significant cross-species commonalities in epileptogenic pathways (Yu et al. 2017). This comparative approach is particularly critical given the high prevalence of drug resistance in canine idiopathic epilepsy; for instance, miR-134 has emerged as a promising circulating biomarker and potential therapeutic target for drug-resistant cases in both humans and dogs (Henshall et al. 2016; Potschka et al. 2023). The implementation of these precise diagnostic and prognostic tools is essential to optimise therapeutic interventions and facilitate the transition toward personalised medicine in veterinary neurology.

1.2.2 Proteomic and inflammatory biomarkers

Proteomic and inflammatory biomarkers detect proteins or cytokines in blood/CSF reflecting neuroinflammation or neuronal damage. For instance, lipid mediators and nitric oxide levels in CSF/plasma distinguish inflammatory conditions from IE (Yonezawa et al. 2024). Furthermore, proteomic analysis of cerebrospinal fluid in human patients with mesial temporal lobe epilepsy has identified increased apolipoprotein AI levels as markers for the extent and severity of central nervous system injury (Baka et al. 2020). In human epilepsy research, comprehensive proteomic profiling has revealed a diverse array of differentially expressed proteins, including apolipoproteins (APOA1, APOD, and APOA4), serpin protease inhibitors (SERPINA3, SERPINF1), and complement components (C9, C8, and C1R), which are significantly upregulated in temporal lobe epilepsy patients and reflect underlying neuroinflammation, intracellular signalling disruptions, lipid metabolism alterations, and oxidative stress (Glazyrin et al. 2024). Additionally, plasma pro-inflammatory cytokines such as interleukin-6, interleukin-1β (IL-1β), and tumour necrosis factor-α are consistently elevated in both temporal lobe and extra-temporal lobe epilepsy compared to healthy controls, with IL-6 levels being particularly useful for differentiating temporal lobe epilepsy from extra-temporal epilepsy (Basnyat et al. 2021; Kobylarek et al. 2019). The American Epilepsy Society and the National Institute of Neurological Disorders and Stroke have identified IL-1β as a potential biomarker of epilepsy severity, especially in drug-resistant temporal lobe epilepsy, where neocortical levels of NFkB and IL-6 are increased and correlate with seizure frequency (Chmielewska et al. 2020). Moreover, circulatory inflammatory mediators, including IL-1β, interferon-γ, high-mobility group box 1, and CD4+CD38+ cells, have been consistently elevated in blood samples from human patients with refractory epilepsy (Potschka et al. 2023). Beyond cytokines, plasma markers of brain injury such as S100B, neuron-specific enolase, glial fibrillary acidic protein, ubiquitin carboxyl-terminal hydrolase L1, myelin basic

protein, and tau have been evaluated as indicators of neuronal damage in epilepsy models and patients (Butera et al. 2026; Kunda et al. 2019). Recent studies have also investigated the association between pro-inflammatory proteins and neurofilament light chain, a known marker of neurodegeneration, in epilepsy patients, revealing complex interrelationships between inflammation and neurodegeneration in active epilepsy (Sarangdhar et al. 2025).

In veterinary medicine, although data on proteomic and inflammatory biomarkers in canine epilepsy remain comparatively limited, emerging evidence increasingly supports heightened neuroinflammatory signalling within the epileptic canine brain (Potschka et al. 2023). Recent advances in serum proteomics have begun to fill this gap: a tandem-mass-tag–based quantitative proteomic analysis identified multiple differentially expressed proteins in the serum of epileptic dogs, including pathways related to immune activation, oxidative stress, and metabolic dysregulation, underscoring the systemic biological alterations accompanying canine epilepsy (Baka et al. 2024). Complementary metabolomic studies of serum and cerebrospinal fluid further demonstrate that metabolite profiles differ between epileptic and healthy dogs, reflecting disruptions in energy metabolism and neurotransmitter-related pathways (Law et al. 2018; Verdoodt et al. 2024). Although direct comparisons between antiseizure-medication responders and non-responders have not yet been reported in dogs, the identification of circulating miR-134 as a biomarker of drug-resistant epilepsy represents a significant step toward molecular stratification of treatment response miR-134 (Potschka et al. 2023). The inflammatory cascade in epilepsy involves rapid release of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α from activated microglia and astrocytes, initiating within the first 30 minutes after seizure activity and contributing to neuronal hyperexcitability and epileptogenesis through cytokine signalling (Choi et al. 2009). Importantly, this neuroinflammatory response can extend beyond the central nervous system into systemic circulation, amplifying chronic inflammation and providing a mechanistic link between central pathological processes and peripheral biomarker detection strategies (Sönmezler et al. 2023). The integration of proteomic, metabolomic, and molecular biomarkers therefore holds considerable promise for improving diagnostic precision and advancing personalised approaches to canine epilepsy.

1.2.3 Metabolomic biomarkers

Metabolomic biomarker profiles of small molecules are altered in epilepsy, supporting early drug-resistant epilepsy prediction alongside clinical factors. In human medicine, metabolic fingerprinting of serum and plasma has identified significant differences between drug-resistant and drug-sensitive patients, often involving impaired bioenergetics, altered lipid metabolism, and disruptions in oxidative stress pathways (Boguszewicz et al. 2019; Murgia et al. 2017). Research has also explored urinary volatile organic compounds as non-invasive biomarkers, suggesting that organic compounds originating from altered lipid

metabolism may serve as diagnostic indicators for conditions like temporal lobe epilepsy (Fujita, Ota, and Kato 2019). Furthermore, human studies have demonstrated that ketogenic dietary interventions, such as medium-chain triglyceride oil, induce measurable metabolic shifts in lipid and ketone levels that correlate with improved seizure control (Lowe et al. 2021; Yu et al. 2022).

In veterinary medicine, metabolomic profiling has become a powerful tool for differentiating canine idiopathic epilepsy from structural and healthy controls. Analysis of canine cerebrospinal fluid using gas chromatography-mass spectrometry has identified glutamic acid as a key biomarker, showing significantly higher levels in dogs with IE compared to both healthy controls and dogs with structural cerebral pathology (Hasegawa et al. 2013). Other relevant CSF markers include alterations in ascorbic acid (Hasegawa et al. 2013) and components of the endocannabinoid system, such as increased anandamide and decreased 2-arachidonoylglycerol (Verdoodt et al. 2024). Beyond CSF, plasma metabolomics in dogs has revealed broader systemic changes in amino acid metabolism, inflammation, and oxidative stress metabolites (Verdoodt et al. 2025), including vitamin B6 (Verdoodt et al. 2025). Additionally, in dogs receiving dietary MCT supplements, candidate biomarkers such as beta-hydroxybutyrate, pyruvate, and specific triacylglycerol ratios have been identified as tools for monitoring therapeutic response and adjusting nutritional interventions (Berk et al. 2022). These molecular insights from both species contribute significantly to delineating pathogenic processes and refining diagnostic algorithms (Pitkänen et al. 2018).

1.2.4 Neuroimaging biomarkers

Neuroimaging biomarkers, including MRI for hippocampal volumetry and PET/SPECT for glucose metabolism or glutamate binding, reveal subtle epileptogenic changes in human temporal lobe epilepsy, with significant translational potential to canine IE despite often normal routine scans (Drobot et al. 2023; Elisevich et al. 2021). In humans, advanced techniques such as functional MRI (fMRI) and diffusion tensor imaging help identify network-level disruptions and connectivity changes (Arefin 2017). Similarly, in veterinary medicine, voxel-based morphometry has revealed statistically significant reductions in grey matter volume in the olfactory bulb, cingulate gyrus, and temporal and occipital lobes of epileptic Beagles (Frank et al. 2018). Functional neuroimaging using F-18-fluoro-deoxy-glucose (F-18-FDG) PET has also successfully identified regions of reduced glucose metabolism in juvenile Lagotto Romagnolo and adult Finnish Spitz dogs, which frequently correlate with the location of EEG abnormalities (Löscher 2022). Furthermore, resting-state fMRI (rs-fMRI) has demonstrated increased connectivity in the anterior default mode network of dogs with idiopathic epilepsy, mirroring connectivity patterns observed in human patients (Beckmann et al. 2021).

1.2.5 Electrophysiological biomarkers

Electrophysiological biomarkers, such as interictal epileptiform discharges on EEG, indicate neuronal hyperexcitability and are crucial for confirming seizure activity (Horváth et al. 2020), although their specificity is sometimes limited (Fernández-Martín et al. 2023). Beyond standard spikes and sharp waves, pathological high-frequency oscillations (pHFOs)—categorised into ripples (80–200 Hz) and fast ripples (> 150–250 Hz)—have emerged as highly specific markers for the epileptogenic zone in both humans and experimental models (Prida, Staba, and Dian 2015; Fabó et al. 2023). Automated identification of these markers using artificial intelligence is currently being explored to improve the localisation of seizure onset zones and refine clinical management (Varatharajah et al. 2018). Given the limitations in specificity of individual markers, the integration of multiple biomarker modalities—combining structural, functional, and electrophysiological data—offers a more comprehensive diagnostic and prognostic assessment for epilepsy (Pitkänen and Łukasiuk 2011).

Biomarker Type	Summary Description
Genetic & Epigenetic Biomarkers	Identify heritable variants or regulatory changes (e.g., ion channel polymorphisms, microRNA dysregulation) that influence epileptogenesis and prognosis. In dogs, gene/exome sequencing supports diagnosis, drug-resistance prediction, and development of personalised treatment strategies.
Proteomic & Inflammatory Biomarkers	Detect alterations in proteins, cytokines, and inflammatory mediators in blood or CSF that reflect neuroinflammation or neuronal injury. Examples include lipid mediators, nitric oxide, and elevated apolipoprotein AI in CSF, which help differentiate idiopathic from inflammatory or structural causes.
Metabolomic Biomarkers	Profile small-molecule metabolites altered during epileptogenesis or chronic epilepsy. Useful for early prediction of drug-resistant epilepsy and distinguishing idiopathic from structural epilepsy in CSF. Provide insight into disrupted metabolic pathways and disease mechanisms.
Neuroimaging Biomarkers	MRI-based volumetry and PET/SPECT metabolic imaging reveal subtle structural or functional abnormalities (e.g., hippocampal atrophy, altered glucose metabolism) even when routine scans appear normal. Offer translational potential for canine IE despite current limitations in clinical availability.
Electrophysiological Biomarkers	EEG-derived markers such as interictal epileptiform discharges and high-frequency oscillations indicate neuronal hyperexcitability or epileptogenic zones. Useful for confirming seizure activity, though specificity varies, and advanced markers remain primarily research tools.
Circulating Molecular Biomarkers (e.g., miRNAs)	Emerging minimally invasive markers measurable in blood or CSF. Circulating miRNAs show promise for differentiating epilepsy types, predicting treatment response, and monitoring disease progression in dogs, addressing gaps left by conventional diagnostic approaches.

Table 2. Summary of the types of biomarkers used in epilepsy research.

These multimodal biomarkers align with epilepsy hypotheses like neuroinflammation and identified network changes in canine IE (Beckmann et al. 2021). However, the development and application of advanced diagnostic tools, including proteomic analyses, are essential for gaining a deeper understanding of the molecular mechanisms underlying epilepsy and for facilitating accurate clinical diagnoses in both human and canine populations (Baka et al. 2020).

The identification of reliable biomarkers is crucial for enhancing diagnostic accuracy, predicting treatment response, and monitoring disease progression in canine idiopathic epilepsy, particularly given the challenges posed by drug resistance (Potschka et al. 2023). These biomarkers can encompass genetic, proteomic, metabolomic, and imaging markers, each offering distinct advantages in characterising the complex aetiology and progression of the disease (Pitkänen et al. 2018).

1.3. MicroRNAs

MicroRNA is a class of small, non-coding RNA molecules that regulate gene expression at the post-transcriptional level, playing critical roles in various physiological and pathological processes, including neurological disorders like epilepsy (García-Gracia et al. 2024). The structure of miRNA molecules typically involves a short, single-stranded RNA sequence that forms a hairpin-like precursor, which is subsequently processed into a mature, functional miRNA by a series of enzymatic steps. MiRNAs exert their regulatory effects by binding to complementary sequences within target messenger RNA (mRNA) molecules, leading to translational repression or mRNA degradation, thereby modulating protein synthesis (Fig.5), (Ma 2017). In the context of epilepsy, alterations in miRNA expression profiles within biofluids, particularly blood, offer a promising avenue for biomarker discovery due to their stability, accessibility, and critical involvement in neuroinflammation and neuronal plasticity (Enright, Simonato, and Henshall 2018). Specific microRNAs (miRNAs) have emerged as promising circulating biomarkers for epilepsy, with studies identifying altered expression profiles in both human patients and animal models that correlate with disease diagnosis, prognosis, and even drug-resistant phenotypes (Ma 2017). This regulatory capacity makes miRNAs highly attractive targets for diagnostic and therapeutic development, as their dysregulation can influence neuronal excitability, synaptic plasticity, and neuroinflammation, all central to epileptogenesis.

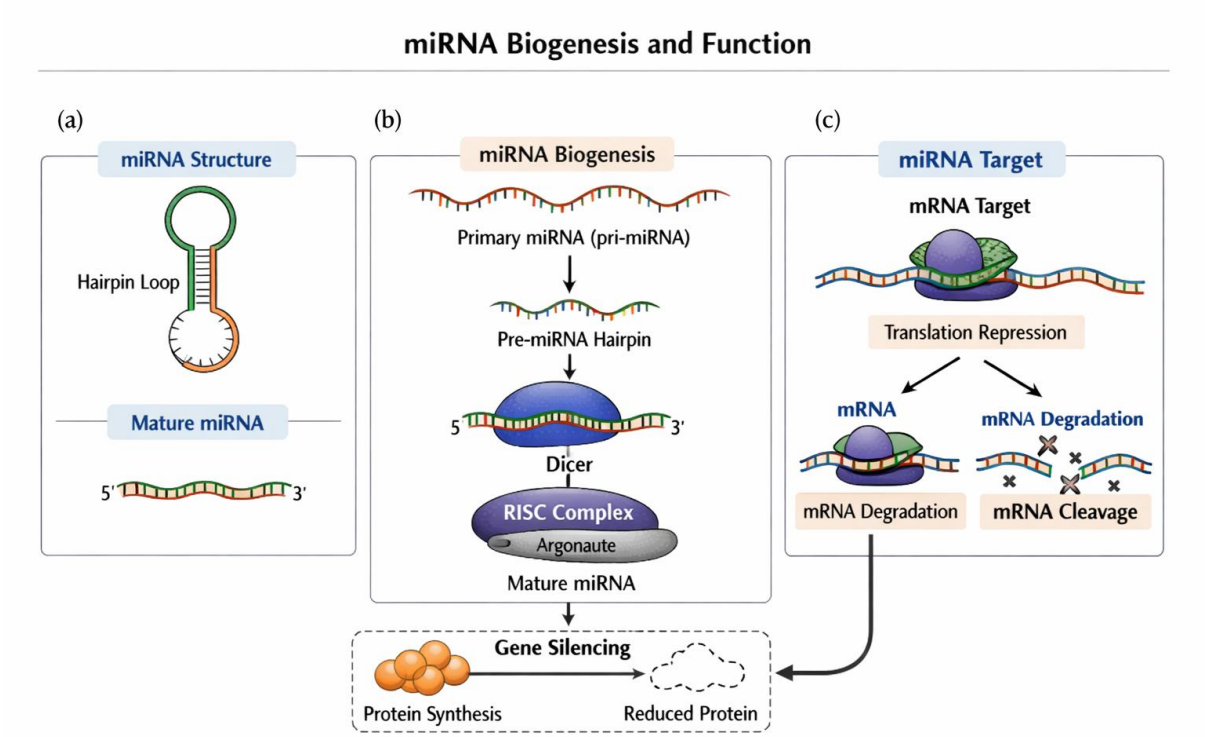


Figure 5. Schematic representation of microRNA (miRNA) biogenesis and mechanism of gene silencing. Primary miRNA (pri-miRNA) transcripts are synthesised by RNA polymerase II and fold into characteristic hairpin structures. These are processed in the nucleus by the Drosha–DGCR8 complex to form precursor miRNA (pre-miRNA), which is exported to the cytoplasm via Exportin-5. The Dicer enzyme cleaves the pre-miRNA hairpin to yield a short double-stranded RNA duplex, from which one strand (the mature miRNA) is incorporated into the RNA-induced silencing complex (RISC) containing Argonaute proteins. The miRNA–RISC complex binds complementary sequences within target messenger RNA (mRNA), leading either to translational repression or mRNA degradation depending on the degree of sequence complementarity. The overall outcome is post-transcriptional gene silencing, reducing protein synthesis from the targeted mRNA (Cha et al. 2017; Libri et al. 2013).

1.3.1 miRNAs in the pathophysiology of epilepsy

MicroRNAs (miRNAs) are fundamental orchestrators in the pathophysiology of epilepsy, modulating complex gene networks that govern neuronal excitability, synaptic remodelling, neuroinflammation, and programmed cell death (Alsharafi et al. 2015; Dogini et al. 2013). Experimental models in this context refer to induced or genetic epilepsy models in rodents, where seizures are triggered using chemoconvulsants such as kainic acid or pilocarpine, electrical stimulation paradigms, or arise spontaneously from defined genetic mutations, thereby recapitulating key molecular features of human epileptogenesis. Several specific miRNAs, including miR-132 and miR-146a, are consistently dysregulated in the brain tissue of both human patients and experimental models (Alsharafi et al. 2015; Henshall 2014).For instance, miR-324-5p is upregulated in hippocampal neurons following seizures, where it directly suppresses the translation of the A-type potassium channel Kv4.2 (encoded by Kcnd2) (Thomas, Groß, and Bassell 2018). Inhibition of miR-324-5p with antagomirs (chemically engineered, single-stranded oligonucleotides designed to silence endogenous miRNAs) has been shown to restore Kv4.2 expression and delay seizure onset, thereby reducing seizure severity and protecting against neurodegeneration (Thomas, Groß, and Bassell 2018).

Similarly, miR-134 levels are elevated in experimental and human epilepsy, where the miRNA influences dendritic spine morphology by targeting Limk1 (Henshall 2014). Silencing miR-134 through the administration of antagomirs significantly reduces spine density, attenuates the severity of status epilepticus, and enhances resistance to evoked seizures, resulting in a reduction in neuronal loss and gliosis (Jiménez-Mateos et al. 2012). miR-146a is critical for regulating astrocyte-mediated inflammatory responses and is consistently upregulated following epileptogenic stimuli, contributing to reactive astrogliosis (Alsharafi et al. 2015). Furthermore, a reduction of miR-128 expression in postnatal neurons causes increased motor activity and fatal epilepsy in mice, underscoring its essential role in maintaining the excitatory/inhibitory balance (Tan et al. 2013). Because miRNAs are stable and can be reliably detected in biofluids such as serum or plasma, they represent promising non-invasive biomarkers for diagnosing epileptogenesis and monitoring treatment response (Henshall 2014). Collectively, these findings establish miRNAs as key molecular regulators of seizure propagation and potential therapeutic targets for disease modification (Dogini et al. 2013).

Functional studies using antagomirs against miRNAs like miR-134 have reduced seizure severity and neuropathology—including neuronal loss and gliosis—while functional studies employing antagomirs indicate these regulatory RNAs as promising targets for new possible strategies in the treatment of epilepsy (Dogini et al. 2013). Their stability in biofluids further establishes miRNAs as ideal candidates for non-invasive diagnostic and prognostic tools in canine epilepsy (García-Gracia et al. 2024).

1.3.2 The role of miRNAs in epileptogenesis

Epileptogenesis, the process by which a normal brain develops epilepsy, involves intricate molecular and cellular alterations, wherein miRNAs play a pivotal role in modulating gene expression networks critical for neuronal plasticity, inflammation, and survival (Henshall 2013). Specifically, dysregulation of miRNAs such as miR-21, miR-34a, miR-132, and miR-146a are frequently observed in animal models of epileptogenesis, influencing processes such as dendritic arborization and astrocyte-mediated inflammatory responses (Pitkänen et al. 2015).

The role of miRNAs in epileptogenesis has garnered increasing attention, as these regulatory molecules orchestrate many molecular and cellular pathways underlying the development of spontaneous recurrent seizures (Wang et al. 2016; Silva et al. 2020). For instance, dysregulation of miRNAs involved in neuroinflammatory signalling—particularly miR-146a and miR-155—reinforces the centrality of chronic inflammation and glial activation in driving epileptogenic remodelling, aligning with evidence from experimental and human epilepsy studies (Alsharafi 2015; Fabene 2010). Similarly, miRNAs that modulate neuronal excitability and synaptic structure, such as miR-134, contribute by altering dendritic spine

morphology and heightening seizure susceptibility; notably, its inhibition reduces seizure activity and neuronal loss in vivo (Henshall 2014). Other miRNAs, including miR-34a and miR-21, govern apoptotic pathways involving BCL2, p53, and caspases, where their dysregulation during epileptogenesis exacerbates neuronal loss, thus promoting network hyperexcitability (Xie et al. 2023),(Hu et al. 2012). miRNAs implicated in synaptic plasticity and circuit reorganisation, such as miR-132, miR-124, and miR-128, further enable aberrant connectivity changes like mossy fibre sprouting, which are hallmarks of the epileptogenic hippocampus (Henshall 2013; McSweeney et al. 2016; Wang and Zhao 2021). Extending into glial function, miRNAs such as miR-146a and miR-223 modulate astrocyte reactivity and glutamate homeostasis, broadening their influence on seizure threshold (Eacker, Dawson, and Dawson 2013; Groß and Tiwari 2018; Iyer et al. 2012). Collectively, these miRNAs act as upstream regulators of neuroinflammation, excitability, apoptosis, and synaptic remodelling—key processes converging to foster a pro-epileptic milieu (Henshall 2014; Wang and Zhao 2021). Coupled with their detectability in peripheral biofluids, this positions them as potent mechanistic biomarkers and therapeutic targets to alter disease progression (Dent and Chen 2022; Ma 2017; Mao 2023). In particular, miR-146a stands out as a promising biomarker for epileptogenesis, prognosis, and treatment resistance, owing to its pivotal role in neuroinflammation and disease-related cellular processes (Dent and Chen 2022; Mao et al. 2023).

1.3.3 miRNAs as potential biomarkers in epilepsy.

The identification of novel, minimally invasive biomarkers for drug-resistant epilepsy holds significant promise for enhancing therapeutic strategies and improving prognosis through early diagnosis and optimised treatment regimens (Wang et al. 2015). Recently, microRNA (miRNA) alterations have emerged as promising candidates due to their stability and detectability in serum, which enables non-invasive diagnostics (Wang et al. 2015). Several studies have demonstrated the utility of circulating miRNAs in distinguishing drug-sensitive from drug-resistant epilepsy, as well as differentiating epilepsy patients from healthy controls. For instance, miR-301a-3p has been identified as a highly sensitive and specific diagnostic biomarker for drug-resistant epilepsy, showing significant downregulation in affected cohorts relative to treatment-responsive patients and healthy controls (Wang and Zhao 2021).

Moreover, more than 165 distinct miRNAs have been observed in epilepsy patients and animal models, particularly those with mesial temporal lobe epilepsy (Korotkov et al. 2017). These findings underscore the potential of miRNAs as non-invasive biomarkers for early detection, prognostic assessment, and personalised interventions in drug-resistant epilepsy (Wang et al. 2015). In canine idiopathic epilepsy, specific miRNAs, including miR-16, miR-93-5p, miR-142, miR-132, and miR-574, have shown potential in differentiating disease subtypes and predicting treatment outcomes (García-Gracia et al. 2024). Dysregulation of miRNAs in key pathophysiological processes, including neuroinflammation, further

supports their promise as both biomarkers and therapeutic targets (Dent and Chen 2022; Xie et al. 2023). Specifically, altered miRNA expression has been linked to hippocampal pathology and seizure activity in human patients and experimental epilepsy models, reflecting intricate gene-expression regulation underlying the disease (Henshall et al. 2016; Roncon et al. 2015). These miRNA changes coincide with characteristic structural abnormalities in the epileptic hippocampus, including selective interneuron and pyramidal cell loss, axonal and dendritic reorganisation, synaptic remodeling, and region-specific vulnerability within the dentate gyrus and CA1–CA3 subfields.

MicroRNAs have gained increasing attention as potential biomarkers for idiopathic epilepsy in humans, owing to their disease-associated expression patterns and mechanistic involvement in epileptogenesis (Moustafa et al. 2020; Xie et al. 2023). Several miRNAs, including miR-134 and miR-146a, exhibit consistent dysregulation in key epileptogenic regions such as the hippocampus and cortex, reflecting alterations in neuronal excitability, synaptic plasticity, and neuroinflammatory pathways (Sumadewi, Harkitasari, and Tjandra 2023; Henshall et al. 2016). Their remarkable stability in biofluids—including blood and cerebrospinal fluid—due to protection within exosomes or protein complexes further supports their suitability for minimally invasive diagnostic approaches (Henshall 2013). Importantly, specific miRNA changes have been detected before the onset of spontaneous seizures in experimental models, suggesting a role in early detection and risk stratification during the preclinical phase of epileptogenesis (Szydłowska et al. 2024). Moreover, miRNA expression profiles may provide insight into treatment response, as levels can shift following antiseizure therapies or between seizure onset and post-seizure states, offering a complementary tool for monitoring disease progression (Sun et al. 2016). Collectively, these characteristics position miRNAs as promising molecular indicators of the subtle network and gene-regulatory disturbances that underlie idiopathic epilepsy (Khemka et al. 2024).

Underpinning this biomarker potential, microRNAs serve as pivotal modulators in the pathophysiology of epilepsy, influencing epileptogenesis through regulation of neuroinflammation, apoptosis, synaptic plasticity, and ion channel expression (Groß and Tiwari 2018; Thomas, Groß, and Bassell 2018; Xie et al. 2023). These approximately 22-nucleotide noncoding RNAs fine-tune gene expression by binding target mRNAs and suppressing translation or inducing degradation (Dogini et al. 2013). In the hippocampus—especially in temporal lobe epilepsy—altered levels of miR-132, miR-134, miR-146a, miR-155, miR-34a, miR-211-5p, and miR-324-5p have been documented in both animal models and patient tissues (Hu et al. 2012),(Dent and Chen 2022; Li et al. 2024). For instance, miR-132 and miR-134 promote dendritic remodelling and hyperexcitability, while elevated miR-146a and miR-155 reflect reactive gliosis and innate immune signalling via TLR and cytokine pathways (Hu et al. 2012),(Jiménez-Mateos et al. 2012),(Iyer et al. 2012),(Vezzani, Lang, and Aronica 2015). Downregulation of miR-211-5p contributes to oxidative stress and ferroptosis through the P2RX7 and ERK–GPX4 signalling axis (Li et al. 2024). Additionally,

miR-324-5p suppresses the Kv4.2 potassium channel, thereby facilitating seizure generation through increased neuronal excitability (McGann et al. 2023), (Thomas, Groß, and Bassell 2018),(Groß and Tiwari 2018). Therapeutically, targeting several of these miRNAs via antagomirs can attenuate seizure-induced neuronal death, neuroinflammation, and the progression of spontaneous recurrent seizures, highlighting their potential as disease-modifying interventions (Jiménez-Mateos et al. 2012; Sumadewi, Harkitasari, and Tjandra 2023).

miRNA	Function / Target	Epilepsy-Related Role	Refs
miR-132	Dendritic remodelling; neuronal plasticity	Dysregulated; promotes hyperexcitability and aberrant sprouting	Alsharafi 2015; Henshall 2014; Henshall 2013
miR-146a	Astrocyte inflammatory signalling	Upregulated; drives reactive astrogliosis; biomarker potential	Alsharafi 2015; Dent & Chen 2022; Mao 2023
miR-324-5p	Suppresses Kv4.2 channel	Upregulated post-seizure; increases excitability; antagomir protective	Thomas 2018; Groß & Tiwari 2018
miR-134	Targets Limk1; spine morphology	Elevated; increases seizure susceptibility; antagomir reduces neuronal loss	Henshall 2014; Jiménez-Mateos 2012
miR-128	Maintains excitatory/inhibitory balance	Reduced expression causes fatal epilepsy; promotes hyperexcitability	Tan 2013
miR-21	Apoptosis (BCL2/p53/caspases)	Dysregulated; promotes neuronal loss during epileptogenesis	Xie 2023; Hu 2012
miR-34a	Apoptotic signalling	Upregulated; contributes to neuronal death and network instability	Xie 2023; Hu 2012
miR-155	Innate immune signalling	Elevated; drives neuroinflammation and glial activation	Alsharafi 2015; Vezzani 2015
miR-124	Synaptic plasticity	Contributes to aberrant connectivity and sprouting	Henshall 2013
miR-223	Astrocyte reactivity; glutamate homeostasis	Modulates seizure threshold	Eacker 2013; Iyer 2012
miR-301a-3p	Circulating biomarker	Downregulated in drug-resistant epilepsy	Wang & Zhao 2021
miR-16	Circulating biomarker	Differentiates canine epilepsy subtypes	García-Gracia 2024
miR-93-5p	Circulating biomarker	Predicts treatment outcomes	García-Gracia 2024
miR-142	Circulating biomarker	Associated with canine idiopathic epilepsy	García-Gracia 2024
miR-574	Circulating biomarker	Differentiates canine epilepsy phenotypes	García-Gracia 2024
miR-211-5p	Oxidative stress; ferroptosis (P2RX7/ERK–GPX4)	Downregulated; promotes oxidative stress and vulnerability	Li 2024

Table 3. MicroRNA dysregulation in epilepsy. This figure highlights key miRNAs involved in neuronal excitability, synaptic remodelling, neuroinflammation, apoptosis, and biomarker development.

1.4 Aims of the thesis

This research aims to identify and validate circulating miRNA signatures that differentiate between canine idiopathic epilepsy and drug-resistant forms, thereby providing a basis for non-invasive diagnostic tools. We hypothesised that dogs with idiopathic epilepsy would exhibit distinct circulating miRNA expression profiles compared to healthy dogs, and that refractory cases could be differentiated based on unique miRNA signatures. Specifically, the study will analyse miRNA expression profiles in plasma collected from distinct canine cohorts to characterise potential diagnostic signatures that distinguish idiopathic cases from those failing to respond to conventional antiseizure medication.

- Identify unique miRNA expression profiles: Determine distinct circulating miRNA signatures that differentiate dogs with idiopathic epilepsy from healthy control animals.
- Differentiate treatment-resistant cases: Identify specific miRNA signatures that can distinguish refractory (drug-resistant) epilepsy from drug-sensitive cases.
- Develop diagnostic and prognostic tools: Evaluate the potential of blood-derived miRNAs as clinically relevant biomarkers for diagnosing idiopathic epilepsy and predicting the likelihood of developing pharmacoresistance.
- Monitor disease progression and severity: Use specific plasma miRNA signatures to indicate disease phases and distinguish between varying seizure frequencies.
- Understand multi-targeting biological pathways: Gain a comprehensive understanding of how miRNAs influence both intended and unintended biological mechanisms through their multi-targeting functions.

2 Materials and methods

2.1 Study design and ethics approval

This cohort prospective study was approved by the Research Ethics Committee of the School of Biodiversity, One Health and Veterinary Medicine of the University of Glasgow (ethics committee approval no.: EA05/19). All clinical protocols were conducted in strict adherence to institutional ethical standards for animal research, ensuring full compliance with veterinary regulatory requirements for the welfare of the canine subjects.

2.2 Dog recruitment

Owners provided informed written consent for study participation and could withdraw this at any time. Dogs were recruited at the University of Glasgow. Plasma samples from seven healthy dogs (obtained from animals admitted to the hospital for routine surgical procedures (ovariectomies/orchiectomies) undergoing routine blood tests) and 14 dogs with a tier II confidence level for idiopathic epilepsy were collected. Amongst epileptic dogs, seven were drug-naive and seven on antiseizure drugs (ASD), of which three were drug resistant (had less than 50% reduction of seizures despite at least two ASD).

Case	Sample Name	Breed	age (months)	Sex	age at first seizure	seizures per month	Cluster seizures	Status epilepticus
Control 1	C1	Cocker Spaniel	12	M	NA	0	0	0
Control 2	C2	Beagle	14	F	NA	0	0	0
Control 3	C3	Border Collie	12	MN	NA	0	0	0
Control 4	C4	Bichon Frise	126	MN	NA	0	0	0
Control 5	C5	Great Dane	37	M	NA	0	0	0
Control 6	C6	Boston Terrier	12	F	NA	0	0	0
Control 7	C7	German Shepherd	94	FN	NA	0	0	0
Naive 1	N1	Border Collie	60	F	60	2	0	0
Naive 2	N2	Labradoodle	60	FN	48	0.5	0	0
Naive 3	N3	Labrador	60	M	58	4	1	0
Naive 4	N4	Beagle	24	FN	24	1.5	0	0
Naive 5	N5	Hungarian Vizla	60	MN	60	3	1	0
Naive 6	N6	Dachshund	60	MN	55	1	0	0
Naive 7	N7	Labrador	48	M	48	1	0	0
Treated 1	T1	Border Collie	24	F	15	2	0	0

Treated 2	T2	Lhasa apso	12	FN	9	6	1	0
Treated 3	T3	JRT	24	MN	21	2	1	0
Treated 4	T4	Border Collie	72	M	60	1	1	0
Treated 5 *	TR1	Boxer	12	MN	9	10	1	1
Treated 6 *	TR2	CKCS	21	MN	18	3	1	0
Treated 7 *	TR3	Border Collie	69	FN	10	2	1	0

Table 3. Summary of animals recruited in the study; * is for refractory cases.

2.3 Sample collection

A formal sample size calculation was not performed. The number of samples included (21) reflected the maximum feasible within the available financial resources, and groups were therefore divided evenly. Blood samples were collected at the presentation and processed within 30 minutes of sampling. Blood samples were allowed to clot for 20 minutes at room temperature. All work surfaces were decontaminated using RNaseZap. Serum was obtained by centrifugation at 2,000 × g for 10 minutes at room temperature. A 200 µL aliquot of the resulting serum was collected for RNA extraction and was aliquoted and stored at –20 °C (for short storage < 2 weeks) and –80 °C (for longer storage).

2.4 RNA Library construction and sequencing

Total RNA was extracted using Trizol reagent (Invitrogen, CA, USA) following the manufacturer's protocols. Total RNA quality and quantity were assessed using a Bioanalyzer 2100 (Agilent, CA, USA). Samples with an RNA integrity number (RIN) of at least 7 were used for further analysis. Approximately 1 ug of total RNA was used to prepare a small RNA library using a TruSeq Small RNA Sample Prep Kits (Illumina, San Diego, USA). The libraries were then single-end sequenced using an Illumina HiSeq 2500 at LC Sciences (Hangzhou, China). Sequencing analysis was performed by the company LC Sciences (Houston, Texas, USA).

2.5 Bioinformatics analysis

For the analysis, ACGT101-mir software (LC Sciences, Houston, Texas, USA) was used to remove adapters, dimers, low complexity and common RNA and repeats from the raw reads. Unique sequences that were between 18 and 26 nucleotides long were annotated with BLAST using miRbase v22.0 (Kozomara, 2019). Variation in lengths at the 3’ and 5’ ends and one sequence mismatch were permitted.

Unique sequences with BLAST results matching mature miRNAs were annotated as known miRNAs, whereas unique sequences with hits to the complementary arm of a known precursor hairpin were instead identified as novel -5p or -3p miRNAs. Putative new and unannotated miRNAs were predicted from unmapped sequences using the BLAST search and RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi; Lorenz 2011). For analysis, miRNA expression values were normalised to the total number of mapped reads in the library and counts per million (CPM). Differential expression was assessed using Welch's t-test for two predefined comparisons: healthy control dogs (n = 7) versus treatment-naïve dogs with epilepsy (n = 7), and treatment-responsive (n = 4) versus treatment-resistant (n = 3) dogs receiving antiseizure medication. Given the exploratory nature of the analysis, unadjusted P values < 0.05 were used to identify candidate miRNAs showing evidence of differential expression. No correction for multiple testing was applied; therefore, these findings were considered exploratory and require validation in an independent cohort. To explore the whole data set, principal component analysis (PCA) and heatmaps were generated using the R environment for statistical computing (https://www.R-project.org/). Principal component analysis (PCA) is an unsupervised multivariate statistical method that reduces the dimensionality of complex datasets by transforming correlated variables into a smaller number of uncorrelated principal components that capture the greatest sources of variation within the data. It was performed on normalised expression data to assess overall sample clustering, identify potential outliers and visualise major sources of variation

2.5 Validation of selected miRNA with RT-qPCR

Among the evaluated miRNAs, we have selected for further validation targets with the highest fold change and the lowest p-value between Naïve Epilepsy vs Control animals (3 miRNAs) and Resistant vs Responder animals (3 miRNAs). Total RNA was extracted from 200 µL of serum using the miRNeasy Serum/Plasma Kit (Qiagen), following the manufacturer's instructions. DNase I was added directly onto the column membrane and incubated for 15 minutes at room temperature. RNA was eluted in 50 µL of RNase-free water. The concentration of microRNA was determined using the Qubit™ microRNA Assay Kit (Thermo Fisher Scientific). Samples were stored at -80°C until further analysis. Poly(A) tailing and cDNA synthesis were performed using the miRNA 1st-Strand cDNA Synthesis Kit (Agilent Technologies) according to the manufacturer's instructions, together with SuperScript™ III Reverse Transcriptase (Invitrogen, 18080044). RT-qPCR was carried out using the Brilliant III Ultra-Fast SYBR QPCR Master Mix (Agilent Technologies, 600882). Reactions were run on the AriaMx Real-Time PCR System (Agilent Technologies) with accompanying software. All RT-qPCR reactions were performed at least in duplicate from four biological replicates. Serum samples derived from healthy individuals were included as controls. The expression of predicted miRNA target genes was normalised to miR-27b expression as the reference gene,

using the formula reported by Pfaffl et al. (2002). Primers were designed based on miRNA sequences and verified its melting temperature using the free software: http://insilico.ehu.es/. Primer efficiency was calculated using standard curves derived from 1:10 serial dilutions of cDNA samples. PCR primer sequences are listed in Supplementary Table ST1. Statistical analyses and plots were generated using the fgStatistics package (Di Rienzo et al., 2012, https://sites.google.com/site/fgstatistics). The selection of miR-27b as a reference gene was based on sequencing data analysis of read counts, and further confirmed by RT-qPCR results, as no variation in Ct values was observed across the different experimental conditions and biological replicates.

3 Results

3.1 RNAseq screening of serum from dogs

To evaluate blood circulating miRNA as a potential biomarker in canine epilepsy, we sequenced miRNA profiles from the blood of dogs diagnosed with idiopathic epilepsy and healthy control animals. We included in the study seven control/healthy dogs and 14 dogs with idiopathic epilepsy (tier II confidence level). Amongst these 14, seven were drug-naive and seven were administered antiseizure drugs (ASD). Three of the ASD dogs were now drug resistant (had less than 50% reduction of seizures despite at least two ASD). An initial examination of the data using principal component analysis suggested that appeared to separate into three groups consisting of controls, drug naïve dogs and drug treated dogs. There was little separation between drug responders and resistant animals (Fig.6).

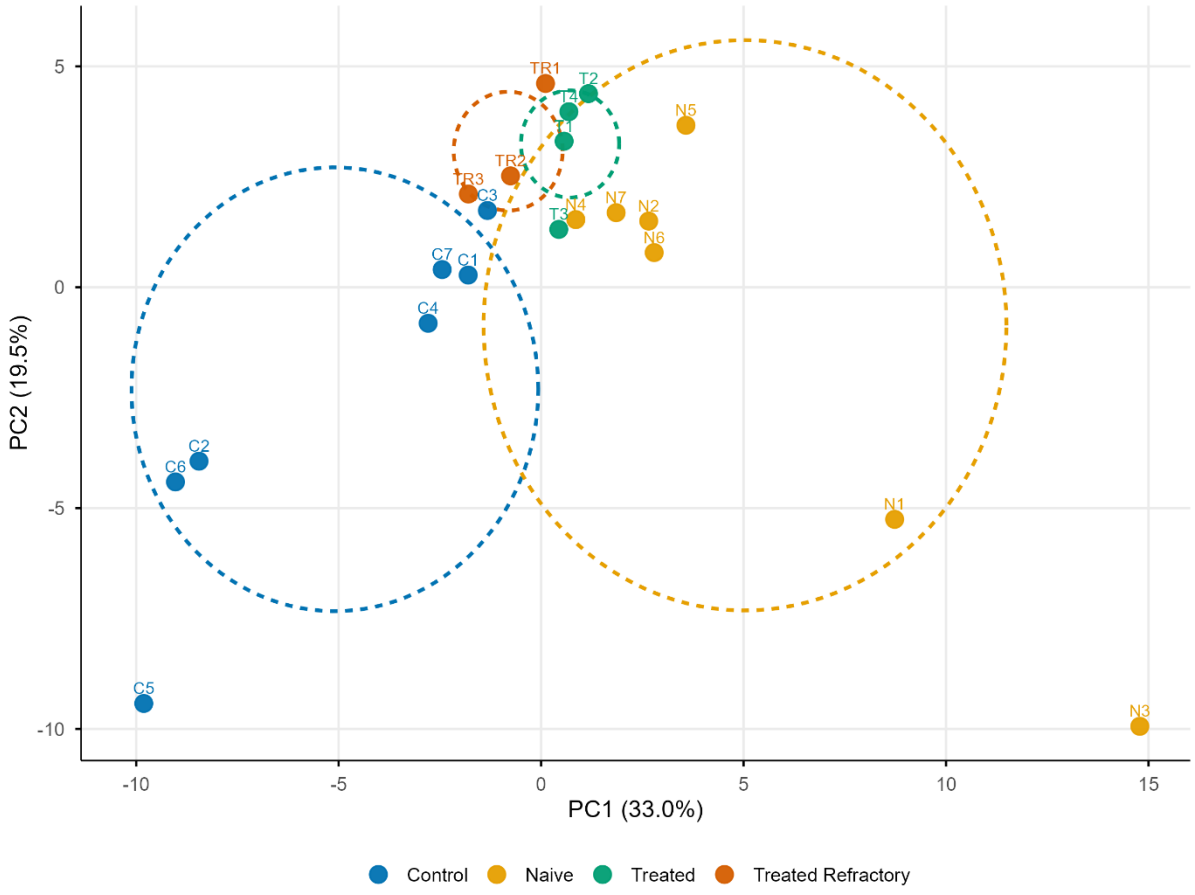


Figure 6: Principal component analysis of CPM-normalised miRNA expression data. Each point represents an individual animal, coloured by group: Control (blue), Drug-naïve epileptic (orange), Treated epileptic responders (green), and Treated refractory epileptic (red). Ellipses represent 95% confidence regions for each group. PC1 and PC2 account for 33.0% and 19.5% of total variance, respectively.

When looking at the specific comparisons of interest, 45 miRNAs were found to significantly differently expressed ($p < 0.05$) when comparing control and drug naïve epileptic animals (Fig.7). Three miRNAs in particular (cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224) showed significant p values < 0.01 and fold

changes > 1.5 between groups. In the drug-resistant vs responder comparison, only six miRNAs were found to be significantly differentially expressed, although again, three had low p values and fold change > 1.5 ((cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224). These six miRNAs were taken forward for further validation using RT-qPCR.

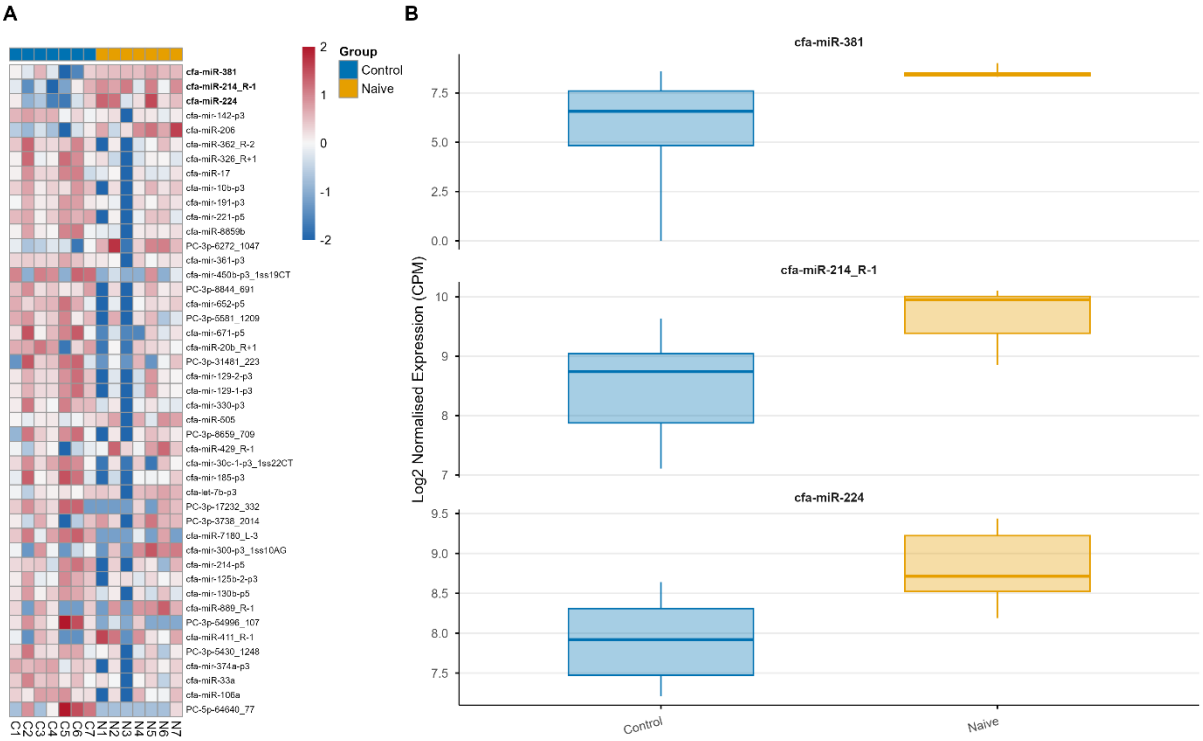


Figure 7.: (A) Heatmap of the significantly expressed miRNAs in Control and Drug-naïve Epileptic dogs, ranked by variance across all samples. Expression values are log2-transformed CPM counts and row-normalised (z-score). Samples are ordered by group (Control, Naive). Row labels for three miRNAs of interest taken forward for further analysis (cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224) are shown in bold (B) Boxplots showing log2 CPM expression of the three miRNAs of interest (cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224) for each group. Boxes show the interquartile range; horizontal line indicates the median. Colours correspond to group: Control (blue), Drug-naïve Epileptic (orange).

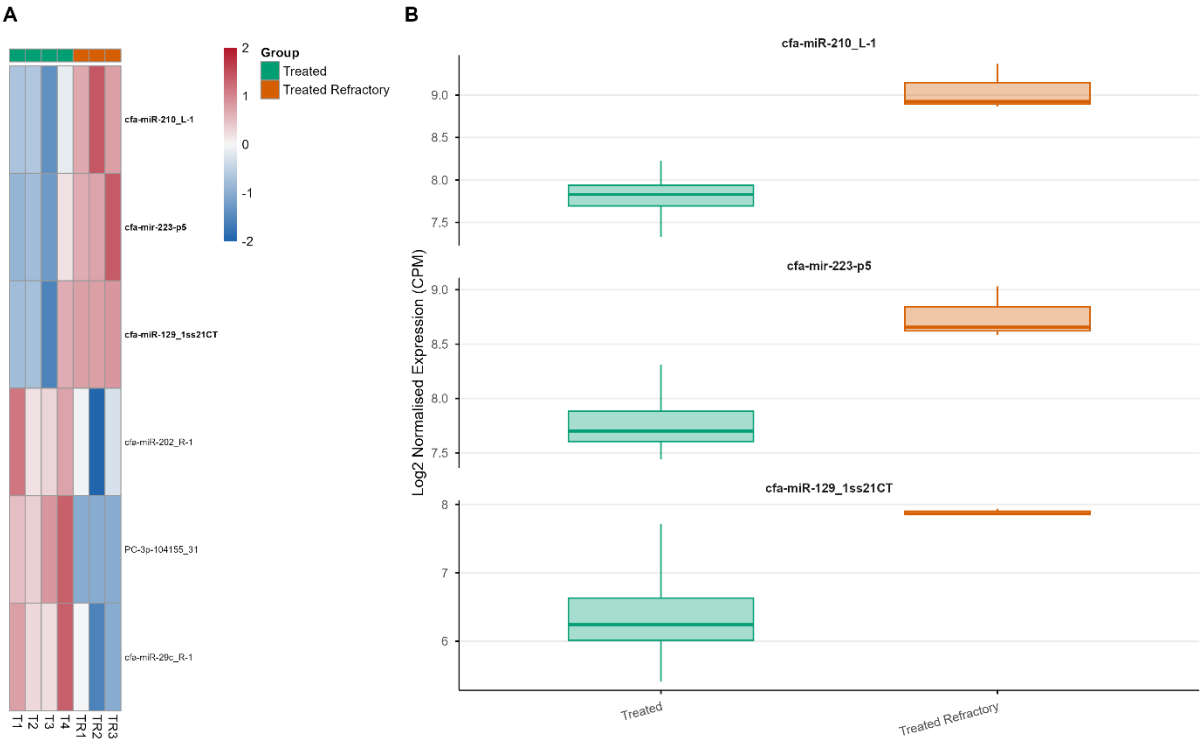


Figure 8.: (A) Heatmap of the significantly expressed miRNAs in Treated Epileptic Responders and Treated Resistant dogs, ranked by variance across all samples. Expression values are log2-transformed CPM counts and row-normalised (z-score). Samples are ordered by group (Control, Naive). Row labels for three miRNAs of interest taken forward for further analysis (cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224) are shown in bold (B) Boxplots showing log2 CPM expression of the three miRNAs of interest (cfa-miR-381, cfa-miR-214_R-1, cfa-miR-224) for each group. Boxes show the interquartile range; the horizontal line indicates the median. Colors correspond to group: Treated (green), Treated Refractory (orange).

3.2 RT-qPCR identification of microRNA biomarkers

Based on sequencing data, six miRNA candidates (cfa miR 381, cfa miR 214, cfa miR 224, cfa miR 210, cfa miR 223, cfa miR 129) were selected for RT qPCR validation. Three miRNAs, including cfa miR 214, cfa miR 223, and cfa miR 210, showed significant differential expression patterns across clinical groups with RT qPCR. The miRNA cfa miR 214 was significantly upregulated in epileptic dogs compared to healthy controls ($p < 0.05$, AUC 0.898), and no clear differences were observed between treatment responsive and treatment resistant animals (Fig. 9). Taken together, moving from sequencing based discovery to qPCR based assay validation indicates that cfa miR 214 has translational potential as a clinically measurable biomarker for epilepsy diagnosis rather than treatment response.

In contrast, cfa-miR-223 expression was significantly reduced in treatment-resistant dogs compared with treatment responders ($p < 0.05$, AUC 0.98), whereas expression levels in healthy and drug-naïve epileptic dogs were similar. This finding differs from the initial RNA-seq results, which suggested increased cfa-miR-223 expression in treatment-resistant animals, and from reports of miR-223 upregulation in drug-resistant human temporal lobe epilepsy (Brennan et al. 2020). Several factors may account for these discrepancies. First, the RNA-seq and RT-qPCR analyses were performed using different normalization

procedures and may differ in their ability to detect low-abundance miRNAs. In addition, the relatively small number of animals, biological variability, and differences in sequencing depth or library composition may have influenced the RNA-seq estimates. Technical variation, including differences in reverse-transcription and primer efficiencies, may also affect qPCR measurements. Species-specific differences in miRNA sequence, target binding, disease mechanisms, treatment history, and circulating miRNA release could further contribute to divergent patterns between canine and human epilepsy. Finally, because the treatment-resistant group was small, the qPCR result should be interpreted cautiously and validated in a larger, independent cohort. Nevertheless, the reduction of cfa-miR-223 detected by qPCR suggests that this miRNA may have potential for distinguishing drug-resistant from treatment-responsive epileptic dogs, although its diagnostic and mechanistic relevance requires further investigation. Finally, cfa-miR-210 was notably upregulated in treatment-resistant animals compared with treatment responders ($p < 0.05$, AUC 0.99) and showed a trend toward higher expression in epileptic dogs relative to healthy controls. These results indicate that miR-210 may be involved both in epilepsy pathogenesis and treatment refractoriness.

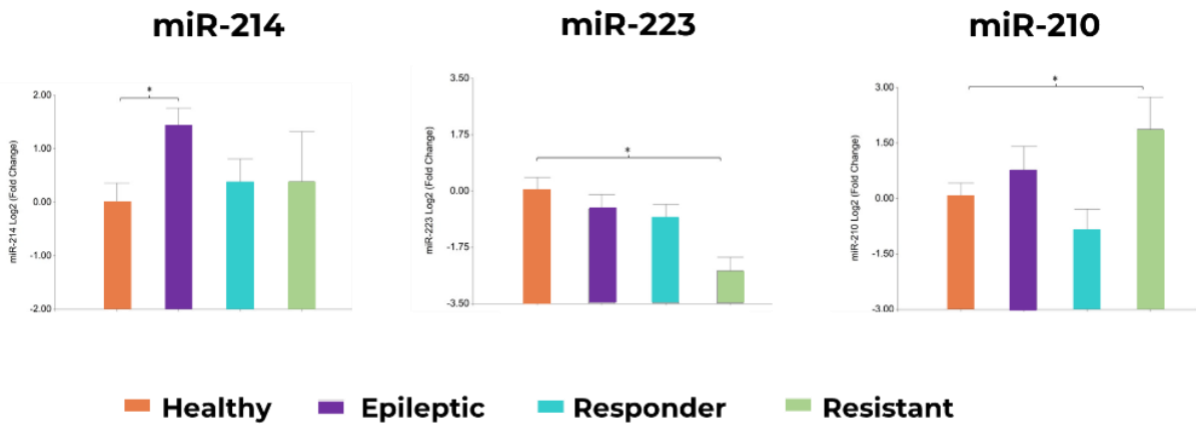


Figure 9. Differential expression of selected miRNAs in canine serum samples assessed by RT-qPCR. Relative expression levels (log_2 fold change) of cfa-miR-214, cfa-miR-223, and cfa-miR-210 in serum samples from healthy dogs (orange, n=7), epileptic dogs (purple, n=7), treatment responders (blue, n=4), and treatment-resistant dogs (green, n=3). (*) indicate statistically significant differences ($p < 0.05$).

4 Discussion

The identification of reliable, non-invasive biomarkers for canine idiopathic epilepsy and the prediction of drug resistance remains a significant unmet need in veterinary neurology. This study provides a potential molecular alternative by identifying circulating microRNA (miRNA) signatures that distinguish healthy dogs from those with IE and differentiate treatment-responsive from treatment-resistant patients. Specifically, cfa-miR-214 was identified as a potential diagnostic marker for epilepsy, exhibiting significant upregulation in epileptic dogs compared to healthy controls, consistent with observations of miR-221-3p in preclinical models distinguishing animals with high and low numbers of seizures (Szydłowska et al. 2023).

4.1 Diagnostic Signatures of Canine Idiopathic Epilepsy

Our screening and validation identified several miRNAs with possible high diagnostic accuracy for IE. Specifically, **cfa-miR-214** (Fold Change 2.08, AUC 0.898), **cfa-miR-381** (FC 2.64, AUC 0.93), and **cfa-miR-224** (FC 1.87, AUC 0.89) were significantly upregulated in drug-naive epileptic dogs compared to healthy controls.

4.1.1 cfa-miR-214: Neurodevelopmental and Inflammatory Modulator

The consistent upregulation of **miR-214** across all epileptic dogs, regardless of treatment status, suggests it may be a robust indicator of the epileptic state rather than a marker of seizure severity or medication response. miR-214 plays critical roles in neural development and inflammatory regulation. It modulates neural progenitor cell differentiation by targeting *Quaking* during cerebral cortex development (Shu, 2017), a process that may be reactivated or dysregulated during epileptogenesis. Additionally, miR-214 participates in mutual regulation with A2AR signalling, playing an important role in the inflammatory response (Zhao, 2015). The upregulation of miR-214 in epileptic dogs may represent a compensatory mechanism attempting to regulate neuroinflammatory processes that contribute to seizure susceptibility, or it may reflect aberrant neurodevelopmental signalling pathways that become re-engaged during epileptogenesis. Further research is warranted to elucidate the precise mechanisms by which cfa-miR-214 contributes to canine epileptogenesis, potentially involving gene targets associated with neuronal excitability or inflammation.

4.1.2 cfa-miR-381: Neural Stem Cell Regulator and Anti-inflammatory Agent

cfa-miR-381 demonstrated the highest diagnostic performance (AUC 0.93) among the identified biomarkers. This miRNA plays important roles in neural stem cell proliferation and differentiation by regulating HES1 expression (Shi et al. 2015). Overexpression of miR-381 promotes neural stem cell proliferation, induces differentiation toward neurons, and inhibits differentiation toward astrocytes (Shi et al. 2015). In ischemic brain injury models, miR-381-3p exerts anti-inflammatory and anti-apoptotic effects by targeting the CCR2/NF-κB pathway, thereby mitigating microglial activation and neurotoxicity (Che et al. 2022). The upregulation of cfa-miR-381 in epileptic dogs may represent a protective response to counteract seizure-induced neuronal damage and inflammation. By suppressing inflammatory mediator expression and reducing neuronal apoptosis (Che et al. 2022), elevated miR-381 levels could reflect the brain's endogenous mechanism to limit excitotoxic damage and maintain neuronal homeostasis. Alternatively, its upregulation may indicate dysregulation of neural stem cell dynamics and neurogenesis processes known to be altered in epilepsy.

4.1.3 cfa-miR-224: Apoptosis Regulator and Signalling Pathway Modulator

cfa-miR-224 participates in multiple biological processes, including cell proliferation, migration, invasion, and apoptosis (Liu et al. 2020). Its mechanisms involve the regulation of the transcription factor SMAD4 (Wang et al. 2013), the miR-224-5p/HOXD10 pathway (Li et al. 2018), AKT activation (Ma et al. 2012), and the NF-κB inflammatory pathway (Li et al. 2013). Notably, inhibition of miR-224-5p restrains neuronal apoptosis by targeting NR4A1 in oxygen-glucose deprivation models (Liu et al. 2020), suggesting that miR-224 may influence neuronal survival pathways. The upregulation of cfa-miR-224 in epileptic dogs could reflect several pathophysiological processes: activation of apoptotic signalling cascades in response to seizure-induced neuronal stress; dysregulation of NF-κB-mediated inflammatory responses that are known to contribute to epileptogenesis or altered AKT signalling pathways that affect neuronal survival and excitability. The involvement of miR-224 in multiple signalling pathways suggests it may serve as an integrative biomarker reflecting the complex molecular changes occurring during epileptogenesis.

4.1.4 Clinical Implications

The high AUC values for these markers support the potential of blood-based miRNAs to complement or eventually refine current diagnostic tiers, offering a rapid, non-invasive, and economical alternative. The distinct but overlapping functions of these three miRNAs—miR-214 in neurodevelopment and inflammation, miR-381 in neural stem cell regulation and miR-224 in apoptosis and signalling pathway modulation suggest they may capture different aspects of the epileptogenic process. Their combined use

could provide a more comprehensive molecular signature of canine idiopathic epilepsy than any single marker alone.

4.2 Molecular Biomarkers of Drug-Resistant Epilepsy

A critical finding of this research is the differentiation of drug-resistant (refractory) epilepsy from drug-sensitive cases. Our data highlighted **cfa-miR-210**, **cfa-miR-223**, and **cfa-miR-129** as significant discriminators between these two clinical groups.

4.2.1 miR-223 and the Excitatory/Inhibitory Balance

In this study, **cfa-miR-223** expression was significantly reduced in treatment-resistant dogs compared to responders ($p < 0.05$). This finding provides an intriguing contrast to human clinical data, where miR-223 has been reported as significantly *upregulated* in patients with temporal lobe epilepsy compared to healthy controls in pilot studies (Benedittis et al. 2021). However, miR-223-3p is known to target the mGluA2 glutamate receptor and the NMDA receptor subunit GluN2B, both of which are critical for regulating neuronal activity (Ge and Wang 2023).

The reduction of miR-223 observed in our refractory canine cohort might indicate a loss of this regulatory control over glutamate receptors, potentially facilitating the hyperexcitability and "breakdown of normal inhibitory processes" characteristic of drug-resistant states (Zhao et al. 2024). Interestingly, miR-223 is decreased in human individuals with hippocampal sclerosis, corroborating a potential role in epilepsy (Groß & Tiwari, 2018). Our results position miR-223 as a valuable candidate for identifying dogs at high risk of treatment failure.

4.2.2 miR-129 and miR-210 as Markers of Refractoriness

Our analysis also identified **cfa-miR-129** (FC 2.35, AUC 0.96) and **cfa-miR-210** (FC 2.35, AUC 0.99) as markers that are significantly upregulated in drug-resistant dogs. **miR-129** is particularly relevant as it has been categorised as an "anti-apoptotic miRNA" in epilepsy research, suggesting its elevation might be part of a cellular response to the recurrent neuronal stress and injury associated with uncontrolled seizures (Xie, et al., 2023). Similarly, the marked upregulation of **miR-210**—frequently termed a "hypoxamiR" due to its induction under low-oxygen conditions—likely reflects the physiological stress and hypoxic conditions resulting from the high seizure density and frequent cluster seizures characteristic of refractory canine epilepsy (Xie, et al., 2023). These findings align with the "intrinsic severity hypothesis," where the biological signatures of seizure burden serve as predictors of medication failure (Santana-Gomez, 2021).

This perspective is further supported by evidence suggesting miR-129 increases seizure susceptibility in rats by repressing the potassium channel Kcna1 (Kalozoumi et al. 2018).

4.3 Comparison with Previous Canine Research

Previous studies in canine IE have highlighted other circulating miRNAs, such as **miR-134**, as biomarkers of drug resistance. Research by García-Gracia et al. also observed significant differences in **miR-16, miR-93-5p,** and **miR-132** levels between drug-sensitive and drug-resistant dogs (García-Gracia, 2024). While our study identified a different subset of miRNAs, the overarching theme remains consistent: drug-resistant dogs exhibit unique circulating molecular profiles that are distinct from those who respond to standard antiseizure medications. The identification of multiple relevant miRNA signatures across different studies underscores the complex, "multifactorial nature" of drug resistance in dogs. This variability across studies may stem from differences in breed populations, epilepsy aetiologies, therapeutic regimens, and methodologies, necessitating further meta-analysis and standardised approaches to identify universally applicable biomarkers.

4.4 Clinical Implications and Future Research

The stability of miRNAs in biofluids and their accessibility via standard blood sampling fulfil key requirements for a clinical biomarker (Enright, Simonato, and Henshall 2018). The signatures identified here—particularly the downregulation of miR-223 and the upregulation of miR-210 and miR-129—could lead to several clinical advancements:

- **Early Stratification**: Identifying dogs predisposed to drug resistance early in the disease course, allowing for earlier implementation of multi-drug protocols or adjunctive therapies.
- **Treatment Monitoring**: Using serum miRNA levels as a "molecular diagnostic" to monitor response to therapy beyond clinical seizure counts.
- **Precision Medicine**: Moving toward personalised treatment strategies that account for the unique molecular pathology of each canine patient.

Future research should focus on validating these candidates in larger, multicentre cohorts and exploring the functional targets of **miR-214** and **miR-210** in canine neural tissue to further elucidate their roles in epileptogenesis.

4.5 Limitations

Despite the promising findings, several limitations within this study warrant consideration. The limitations of this study are multifaceted, beginning with a restricted sample size of only 21 dogs—including 14 with idiopathic epilepsy and a mere 3 in the drug-resistant subgroup—which significantly reduces the statistical power and generalizability of the findings. Although the methodology utilised Tier II diagnostic confidence, the absence of Tier III electroencephalography means that the recorded seizure activity lacked definitive electrophysiological confirmation, leaving open the possibility that some reported events were actually non-epileptic paroxysmal disorders. Furthermore, the inclusion of both drug-naive and treated dogs (7 out of 14) introduces significant confounding variables, as antiseizure medications can influence miRNA expression and modify metabolic pathways. Genetic confounding is also a high risk due to the lack of breed-specific matching; this is a critical oversight as epilepsy phenotypes and treatment responses are known to vary significantly across different breeds. Moreover, the identified miRNA signatures remain purely correlational and require experimental functional validation—such as through the use of antagomirs—to confirm causative roles in epileptogenesis rather than being mere indicators of a disease state (Jiménez-Mateos 2012). Another limitation of our study is that miRNA quantification was performed on free-circulating miRNAs in whole blood rather than on exosome-enriched fractions. Because many epilepsy-associated miRNAs are preferentially packaged within exosomes, analysing only the free miRNA pool may underestimate CNS-derived signals and dilute disease-specific patterns. Exosomal miRNA cargo is increasingly recognised as a more faithful representation of neuronal and glial molecular activity, suggesting that future work incorporating exosome-specific miRNA profiling could improve biomarker sensitivity and mechanistic interpretability. Finally, the study's reliance on a single-point blood collection fails to capture the dynamic temporal fluctuations of miRNAs, which can vary greatly between ictal and interictal phases and throughout the progression of the disease (Enright, 2018).

4.6 Future directions

Future research should prioritise multicentre studies with expanded sample sizes to enhance the statistical robustness and generalizability of identified miRNA biomarkers. These larger cohorts should ideally incorporate diverse breeds, account for potential confounding factors such as anti-seizure medication use, and include more robust diagnostic criteria, such as electroencephalography and comprehensive seizure semiology, to better define epilepsy subtypes and treatment responses. Moreover, longitudinal studies tracking miRNA expression changes over time would provide invaluable insights into the dynamic nature of these biomarkers, particularly in relation to seizure frequency, severity, and response to therapeutic interventions.

5 Conclusion

This study represents initial findings that indicate cfa-miR-214 is a potential diagnostic biomarker for canine idiopathic epilepsy, while a combined signature of reduced miR-223 and elevated miR-210/miR-129 may accurately distinguish drug-resistant from treatment-responsive cases. These findings, if confirmed on a larger cohort, may support the transition toward molecular-based diagnostics in veterinary neurology, offering the potential for more accurate prognosis and the development of targeted, individualised therapeutic approaches for epileptic dogs. The integration of such miRNA profiling with other diagnostic modalities, such as electroencephalography and advanced neuroimaging, could further refine the diagnostic accuracy and prognostic capabilities for canine epilepsy.

References

Abe, Tomohide, Takayuki Seo, Takateru Ishitsu, Takehiro Nakagawa, Masaharu Hori, and Kazuko Nakagawa (2008), "Association between SCN1A polymorphism and carbamazepine-resistant epilepsy," British Journal of Clinical Pharmacology, 66 (2), 304.

Aderinto N, Olatunji G, Kokori E, et al. miR-210 in ischaemic stroke: biomarker potential, challenges and future perspectives. Eur J Med Res. 2024;29(1):432.

Agarwal A, Kaur R, Ashfaque F, et al. Precision medicine approaches in epilepsy: a systematic review of genetic markers and personalized treatment strategies. Precision Med Sci. 2024;13(2):72.

Ahmad, Anas, Mohammad Imran, and Haseeb Ahsan (2023), "Biomarkers as Biomedical Bioindicators: Approaches and Techniques for the Detection, Analysis, and Validation of Novel Biomarkers of Diseases," Pharmaceutics, 15 (6), 1630.

Aícua-Rapún, Irene, Pascal André, and Jan Nový (2018), "Closed-loop Neuropharmacology for Epilepsy: Distant Dream or Future Reality?," Current Neuropharmacology, Bentham Science Publishers.

Alsharafi, Walid A., Bo Xiao, Mutasem Abuhamed, and Zhaohui Luo (2015), "miRNAs: biological and clinical determinants in epilepsy," Frontiers in Molecular Neuroscience, Frontiers Media.

Alkhazaali-Ali Z, Sahab-Negah S, Boroumand AR, Tavakol-Afshari J. MicroRNA as a biomarker for diagnosis, prognosis, and therapeutic molecules in neurodegenerative disease. Biomed Pharmacother. 2024;177:116899.

Alshehri RS, Alrawaili MS, Zawawi BMH, Alzahrany M, Habib AH. Pathophysiology of Status Epilepticus Revisited. Int J Mol Sci. 2025;26(15):7502. Published 2025 Aug 3. doi:10.3390/ijms26157502.

Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011;29(4):341-345.

Arefin, Tanzil Mahmud (2017), "GPR88 signatures in mouse neuronal connectivity and behavior : a potential therapeutic target for psychiatric disorders," HAL (Le Centre pour la Communication Scientifique Directe).

Aronica E, Fluiter K, Iyer A, et al. Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur J Neurosci. 2010;31(6):1100-1107.

Avansini SH, de Sousa Lima BP, Secolin R, et al. MicroRNA hsa-miR-134 is a circulating biomarker for mesial temporal lobe epilepsy. PLoS One. 2017;12(4):e0173060.

Bahlakeh G, Gorji A, Soltani H, Ghadiri T. MicroRNA alterations in neuropathologic cognitive disorders with an emphasis on dementia: lessons from animal models. J Cell Physiol. 2021;236(2):806-823.

Baka R, Eckersall PD, Horvatić A, et al. Quantitative proteomics of cerebrospinal fluid using tandem mass tags in dogs with recurrent epileptic seizures. J Proteomics. 2020;231:103997.

Baka RD, Kuleš J, Beletić A, et al. Quantitative serum proteome analysis using tandem mass tags in dogs with epilepsy. J Proteomics. 2024;290:105034. doi:10.1016/j.jprot.2023.105034.

Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-297.

Basnyat, Pabitra, Soila Järvenpää, Jani Raitanen, Marko Pesu, Kai Lehtimäki, and Jukka Peltola (2021), "A 1-year follow-up study on immunological changes following deep brain stimulation in patients with epilepsy," Scientific Reports, 11 (1).

Bazhanova ED, Kozlov AA, Litovchenko AV. Mechanisms of Drug Resistance in the Pathogenesis of Epilepsy: Role of Neuroinflammation. A Literature Review. *Brain Sci*. 2021;11(5):663. Published 2021 May 19. doi:10.3390/brainsci11050663.

Beckmann, Katrin, Adriano Wang-Leandro, Henning Richter, Rima N. Bektas, Frank Steffen, Matthias Dennler, Inés Carrera, and Sven Haller (2021), "Increased resting state connectivity in the anterior default mode network of idiopathic epileptic dogs," Scientific Reports, 11 (1).

Berendt, Mette, Robyn Farquhar, Paul J. J. Mandigers, A. Pakozdy, Sofie Bhatti, Luisa De Risio, Andrea Fischer, Sam Long, Kaspar Matiasek, Karen R. Muñana, Edward E. Patterson, Jacques Penderis, Simon R. Platt, Michael Podell, Heidrun Potschka, Martí Pumarola, Clare Rusbridge, Veronika M. Stein, Andrea Tipold, and Holger A. Volk (2015), "International veterinary epilepsy task force consensus report on epilepsy definition, classification and terminology in companion animals," BMC Veterinary Research, 11 (1).

Berk, Benjamin-Andreas, Claudia Ottka, Tsz Hong Law, Rowena M. A. Packer, Annette Wessmann, Andrea Bathen-Nöthen, Tarja S. Jokinen, Anna Knebel, Andrea Tipold, Hannes Lohi, and Holger A. Volk (2022), "Metabolic fingerprinting of dogs with idiopathic epilepsy receiving a ketogenic medium-chain triglyceride (MCT) oil," Frontiers in Veterinary Science, 9.

Bergman P, Piket E, Khademi M, et al. Circulating miR-150 in CSF is a novel candidate biomarker for multiple sclerosis. Neurol Neuroimmunol Neuroinflamm. 2016;3(3):e219.

Bhatti SF, De Risio L, Muñana K, et al. International Veterinary Epilepsy Task Force consensus proposal: medical treatment of canine epilepsy in Europe. BMC Vet Res. 2015;11:176.

Boguszewicz, Łukasz, Ewa Jamroz, Mateusz Ciszek, Ewa Emich-Widera, Marek Kijonka, Tomasz Banasik, Agnieszka Skorupa, and Maria Sokół (2019), "NMR-based metabolomics in pediatric drug resistant epilepsy – preliminary results," Scientific Reports, 9 (1).

Brennan GP, Henshall DC. MicroRNAs as regulators of brain function and targets for treatment of epilepsy. Nat Rev Neurol. 2020;16(9):506-519.

Brennan GP, Bauer S, Engel T, et al. Genome-wide microRNA profiling of plasma from three different animal models identifies biomarkers of temporal lobe epilepsy. Neurobiol Dis. 2020;144:105048.

Butera, Ambra, Simona D'Antoni, Antonio Gennaro Nicotera, Maria Vincenza Catania, Carla Consoli, Graziana Ceraolo, Giulia Spoto, Maria Ludovica Albertini, Gabriella Di Rosa, and Elia Maurizio (2026), "Neurodegeneration Biomarkers in Epilepsy: A Critical Review of Current Findings," Life, 16 (2), 296.

Cagnotti, Giulia, Sara Ferrini, Ugo Ala, Claudio Bellino, Cristiano Corona, Elena Dappiano, Giorgia Di Muro, Barbara Iulini, Ida Pepe, Silvia Roncone, and Antonio D'Angelo (2020), "Analysis of Early Assessable Risk Factors for Poor Outcome in Dogs With Cluster Seizures and Status Epilepticus," Frontiers in Veterinary Science, 7.

Campbell A, Morris G, Heller JP, et al. Antagomir-mediated suppression of microRNA-134 reduces kainic acid-induced seizures in immature mice. Sci Rep. 2021;11(1):340.

Cattani AA, Allene C, Seifert V, Rosenow F, Henshall DC, Freiman TM. Involvement of microRNAs in epileptogenesis. Epilepsia. 2016;57(7):1015-1026.

Cavarsan, Clarissa F., Jackeline Moraes Malheiros, Clement Hamani, Imad Najm, and Luciene Covolan (2018), "Is Mossy Fiber Sprouting a Potential Therapeutic Target for Epilepsy?," Frontiers in Neurology, Frontiers Media.

Cha, Wenzhang, Rengen Fan, Yufeng Miao, Yong Zhou, Chenglin Qin, Xiangxiang Shan, Xinqiang Wan, and Ting Cui (2017), "MicroRNAs as novel endogenous targets for regulation and therapeutic treatments," MedChemComm, Royal Society of Chemistry.

Charalambous, Marios, Andrea Fischer, Heidrun Potschka, Matthew C. Walker, Robrecht Raedt, Kristl Vonck, Paul Boon, Hannes Lohi, Wolfgang Löscher, Gregory A. Worrell, Tosso Leeb, Andrew W. McEvoy, Pasquale Striano, Gerhard Kluger, Aristea S. Galanopoulou, Holger A. Volk, and Sofie Bhatti (2023), "Translational veterinary epilepsy: A win-win situation for human and veterinary neurology," The Veterinary Journal, 293, 105956.

Chen L, Zheng H, Zhang S. Involvement of upregulation of miR-210 in a rat epilepsy model. Neuropsychiatr Dis Treat. 2016;12:1731-1737.

Chmielewska, Natalia, Piotr Maciejak, Bartosz Osuch, Miron B. Kursa, and Janusz Szyndler (2020), "Pro-inflammatory cytokines, but not brain- and extracellular matrix-derived proteins, are increased in the plasma following electrically induced kindling of seizures," Pharmacological Reports, 73 (2), 506.

Choi, Jieun, Douglas R. Nordli, Tord D. Alden, Arthur J. DiPatri, Linda Laux, Kent R. Kelley, Joshua M. Rosenow, Stephan Schuele, Veena Rajaram, and Sookyong Koh (2009), "Cellular injury and neuroinflammation in children with chronic intractable epilepsy," Journal of Neuroinflammation, 6 (1).

Chouchi, Malek, Wajih Kaabachi, Hédia Klaa, Kalthoum Tizaoui, Ilhem Ben-Youssef Turki, and Lamia Hila (2017), "Relationship between ABCB1 3435TT genotype and antiepileptic drugs resistance in Epilepsy: updated systematic review and meta-analysis," BMC Neurology, BioMed Central.

Costanza, Carola, Margherita Siciliano, Beatrice Gallai, Rimma Gamirova, and Marco Carotenuto (2025), "Epilepsy and Sleep," in IntechOpen eBooks, IntechOpen.

Cui, Ze-Jia, Ye-Mao Liu, Qiang Zhu, Jingbo Xia, and Hongyu Zhang (2017), "Exploring the pathogenesis of canine epilepsy using a systems genetics method and implications for anti-epilepsy drug discovery," Oncotarget, 9 (17), 13181.

Cardenas-Rodriguez, Noem, Liliana Carmona-Aparicio, Diana Perez-Lozano, Daniel Ortega-Cuéllar, Sael Gomez-Manzo, and Iván Ignacio-Mejía (2020), "Genetic variations associated with pharmacoresistant epilepsy (Review)," Molecular Medicine Reports, Spandidos Publishing.

De Benedittis S, Fortunato F, Cava C, et al. Circulating microRNA as potential diagnostic biomarkers to predict drug resistance in temporal lobe epilepsy: a pilot study. Int J Mol Sci. 2021;22(2):702.

Dent ZO, Chen L. microRNA-146a: a biomarker for epileptogenesis, epilepsy prognosis, and treatment resistance. Open Biomarkers J. 2022;13(1).

Díaz-Coránguez, Mónica, Jerónimo Auzmendi, Monserrat Fuentes-Mejía, Tania Guadalupe Montero Cosme, Alberto Lazarowski, and Luísa Rocha (2024), "Barriers of the CNS and Their Contribution to Drug-Resistant Epilepsy," in AAPS introductions in the pharmaceutical sciences, Springer International Publishing, 181.

Dogini DB, Avansini SH, Vieira AS, Lopes-Cendes I. MicroRNA regulation and dysregulation in epilepsy. Front Cell Neurosci. 2013.

Drobot, Paulina, Marcin Wrzosek, Aleksandra Banasik, Karolina Barbara Owsińska-Schmidt, and Przemysław Podgórski (2023), "Correlation between automated volumetry of temporal lobes and electroencephalography in dogs with idiopathic epilepsy," Research Square (Research Square).

Eacker, Stephen, Ted M. Dawson, and Valina L. Dawson (2013), "The interplay of microRNA and neuronal activity in health and disease," Frontiers in Cellular Neuroscience, 7.

Elisevich, Kost, Esmaeil Davoodi-Bojd, John G. Heredia, and Hamid Soltanian-Zadeh (2021), "Prospective Quantitative Neuroimaging Analysis of Putative Temporal Lobe Epilepsy," Frontiers in Neurology, 12.

Engel, Jerome (2011), "Biomarkers in Epilepsy: Introduction," Biomarkers in Medicine, 5 (5), 537.

Enright, N., Simonato, M. and Henshall, D.C. (2018), Discovery and validation of blood microRNAs as molecular biomarkers of epilepsy: Ways to close current knowledge gaps. Epilepsia Open, 3: 427-436.

Estey, Chelsie M., Curtis W. Dewey, Mark Rishniw, David Lin, Jennifer Bouma, Joseph Sackman, and Erica Burkland (2017), "A Subset of Dogs with Presumptive Idiopathic Epilepsy Show Hippocampal Asymmetry: A Volumetric Comparison with Non-Epileptic Dogs Using MRI," Frontiers in Veterinary Science, 4.

Fabene, Paolo Francesco (2010), "Non-neuronal cells, inflammation and epilepsy (Commentary on Aronica et al.)," European Journal of Neuroscience, Wiley.

Fabó, Dániel, Virag Bokodi, Johanna-Petra Szabó, Emília Tóth, Pariya Salami, Corey J. Keller, Boglárka Hajnal, Thomas Thesen, Orrin Devinsky, Werner Doyle, Ashesh D. Mehta, Joseph R. Madsen, Emad Eskandar, Lóránd Erőss, István Ulbert, Eric Halgren, and Sydney S. Cash (2023), "The role of superficial and deep layers in the generation of high frequency oscillations and interictal epileptiform discharges in the human cortex," Scientific Reports, 13 (1).

Fattorusso, Antonella, Sara Matricardi, Elisabetta Mencaroni, Giovanni Battista Dell'Isola, Giuseppe Di Cara, Pasquale Striano, and Alberto Verrotti (2021), "The Pharmacoresistant Epilepsy: An Overview on Existent and New Emerging Therapies," Frontiers in Neurology, Frontiers Media.

Feng Y, Yang H, Yue Y, Tian F. MicroRNAs and target genes in epileptogenesis. Epilepsia. 2020;61(10):2086-2096.

Fenner, William R. and J.A. Hass (1992), "Mechanisms of seizure disorders.," PubMed, 1 (4), 501.

Fernández-Martín, Raquel, Odile Feys, Elodie Juvené, Alec Aeby, Charline Urbain, Xavier De Tiège, and Vincent Wens (2023), "Towards the automated detection of interictal epileptiform discharges with magnetoencephalography," Journal of Neuroscience Methods, 403, 110052.

Frank, Lisa, Matthias Lüpke, Draginja Kostic, Wolfgang Löscher, and Andrea Tipold (2018), "Grey matter volume in healthy and epileptic beagles using voxel-based morphometry – a pilot study," BMC Veterinary Research, 14 (1).

Fujita, Akiko, Manami Ota, and Keiko Kato (2019), "Urinary volatile metabolites of amygdala-kindled mice reveal novel biomarkers associated with temporal lobe epilepsy," Scientific Reports, 9 (1).

Galanopoulou AS, Moshé SL. In search of epilepsy biomarkers in the immature brain: goals, challenges and strategies. Biomark Med. 2011;5(5):615.

García-Gracia M, Moreno-Martínez L, Hernaiz A, et al. Analysis of plasma-derived exosomal microRNAs as potential biomarkers for canine idiopathic epilepsy. Animals. 2024;14(2):252.

Garg, Nitika, Rupa Joshi, and Bikash Medhi (2020), "A novel approach of targeting refractory epilepsy: Need of an hour," Brain Research Bulletin, 163, 14

Glazyrin, Yury E., Dmitry V. Veprintsev, Elena E. Timechko, Zoran Minić, Tatiana N. Zamay, Д. В. Дмитренко, Maxim V. Berezovski, and Anna S. Kichkailo (2024), "Comparative Proteomic Profiling of Blood Plasma Revealed Marker Proteins Involved in Temporal Lobe Epilepsy," International Journal of Molecular Sciences, 25 (14), 7935.

Guan W, Xu DW, Ji CH, et al. Hippocampal miR-206-3p participates in the pathogenesis of depression via regulating BDNF. Pharmacol Res. 2021;174:105932.

Granata, Tiziana, Nicola Marchi, Erin F. Carlton, Chaitali Ghosh, Jorge González-Martínez, Andreas V. Alexopoulos, and Damir Janigro (2009), "Management of the patient with medically refractory epilepsy," Expert Review of Neurotherapeutics, Taylor & Francis.

Griffin S, Stabile F, De Risio L. Cross-sectional survey of canine idiopathic epilepsy management in primary care in the United Kingdom. Front Vet Sci. 2022;9:907313.

Gross C, Yao X, Engel T, et al. MicroRNA-mediated downregulation of the potassium channel Kv4.2 contributes to seizure onset. Cell Rep. 2016;17(1):37-45.

Hamers MFN, Plonek M, Bhatti SFM, et al. Quality of life in dogs with idiopathic epilepsy and their owners with an emphasis on breed. Front Vet Sci. 2023;9:1107315.

Haqqani AS, Delaney CE, Tremblay TL, et al. Method for isolation and molecular characterization of extracellular microvesicles released from brain endothelial cells. Fluids Barriers CNS. 2013;10(1):4.

Hartz, Anika M. S., Anton Pekcec, Emma L. B. Soldner, Yu Zhong, Juli Schlichtiger, and Bjoern Bauer (2017), "P-gp Protein Expression and Transport Activity in Rodent Seizure Models and Human Epilepsy," Molecular Pharmaceutics, 14 (4), 999.

Hasegawa, Tetsuya, Maho SUMITA, Yusuke Horitani, Reo TAMAI, Katsuhiro Tanaka, Masayuki Komori, and Shigeo Takenaka (2013), "Gas Chromatography-Mass Spectrometry-Based Metabolic Profiling of Cerebrospinal Fluid from Epileptic Dogs," Journal of Veterinary Medical Science, 76 (4), 517.

He Z, Chen H, Zhong Y, et al. MicroRNA-223 targeting ATG16L1 affects microglial autophagy in the kainic acid model of temporal lobe epilepsy. Front Neurol. 2021;12:704550.

Henshall DC. MicroRNAs in the pathophysiology and treatment of status epilepticus. Front Mol Neurosci. 2013;6:37.

Henshall DC, Hamer HM, Pasterkamp RJ, et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. Lancet Neurol. 2016;15(13):1368-1376.

Horváth, András, Emoke Anna Csernus, Sara Lality, Rafał M. Kamiński, and Anita Kamondi (2020), "Inhibiting Epileptiform Activity in Cognitive Disorders: Possibilities for a Novel Therapeutic Approach," Frontiers in Neuroscience, Frontiers Media.

Hu, Kai, Yuanyuan Xie, Chen Zhang, Dongsheng Ouyang, Hongyu Long, Danni Sun, Lili Long, Li Feng, Yi Li, and Bo Xiao (2012), "MicroRNA expression profile of the hippocampus in a rat model of temporal lobe epilepsy and miR-34a-targeted neuroprotection against hippocampal neurone cell apoptosis post-status epilepticus," BMC Neuroscience, 13 (1).

Huang C, You Z, He Y, et al. Combined transcriptomics and proteomics forecast analysis for potential biomarker in the acute phase of temporal lobe epilepsy. Front Neurosci. 2023;17:1145805.

Hussein M, Magdy R. MicroRNAs in central nervous system disorders: current advances in pathogenesis and treatment. Egypt J Neurol Psychiatry Neurosurg. 2021;57:36.

Hülsmeyer, Velia-Isabel, Andrea Fischer, Paul J. J. Mandigers, Luisa De Risio, Mette Berendt, Clare Rusbridge, Sofie Bhatti, Ákos Pákozdy, Edward E. Patterson, Simon R. Platt, Rowena M. A. Packer, and Holger A. Volk (2015), "International Veterinary Epilepsy Task Force's current understanding of idiopathic epilepsy of genetic or suspected genetic origin in purebred dogs," BMC Veterinary Research, 11 (1).

Iyer, Anand M., Emanuele Zurolo, Avanita S. Prabowo, Kees Fluiter, Wim G.M. Spliet, Peter C. van Rijen, Jan A. Gorter, and Eleonora Aronica (2012), "MicroRNA-146a: A Key Regulator of Astrocyte-Mediated Inflammatory Response," PLoS ONE, 7 (9).

Jiang Y, Li L, Tan X, et al. miR-210 mediates vagus nerve stimulation-induced antioxidant stress and anti-apoptosis reactions following cerebral ischemia/reperfusion injury in rats. J Neurochem. 2015;134(1):173-181.

Jiménez-Mateos EM, Henshall DC. Epilepsy and microRNA. Neuroscience. 2013;238:218-227.

Kajin F, Meyerhoff N, Charalambous M, Volk HA. "Resistance is futile": a pilot study into pseudoresistance in canine epilepsy. Animals. 2023;13(19):3125.

Khemka, Niraj, Gareth Morris, Laleh Kazemzadeh, Lara S. Costard, Valentin Neubert, Sebastian Bauer, Felix Rosenow, Morten T. Venø, Jørgen Kjems, David C. Henshall, Jochen H.M. Prehn, and Niamh M. C. Connolly (2024), "Integrative network analysis of miRNA-mRNA expression profiles during epileptogenesis in rats reveals therapeutic targets after emergence of first spontaneous seizure," Scientific Reports, 14 (1).

Kinsey, Nathan, Janelle M. Belanger, and Anita M. Oberbauer (2024), "Differential Gene Expression Associated with Idiopathic Epilepsy in Belgian Shepherd Dogs," Genes, 15 (11), 1474.

Kobylarek D, Iwanowski P, Lewandowska Z, et al. Advances in the potential biomarkers of epilepsy. Front Neurol. 2019;10:685.

Koestner, A. (1992), “Neuropathology of canine epilepsy.,” PubMed, 1 (4), 516.

Korotkov A, Broekaart DWM, Banchaewa L, et al. microRNA-132 is overexpressed in glia in temporal lobe epilepsy and reduces expression of pro-epileptogenic factors in human astrocytes. Glia. 2020;68(1):60-75.

Koskinen, Lotta L. E., Eija H. Seppälä, Jutta Weißl, Tarja S. Jokinen, Ranno Viitmaa, Reetta Hänninen, Pascale Quignon, Andrea Fischer, Catherine André, and Hannes Lohi (2017), “ADAM23 is a common risk gene for canine idiopathic epilepsy,” BMC Genomic Data, 18 (1).

Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res*. 2019;47(D1):D155-D162. doi:10.1093/nar/gky1141.

Kriechbaumer SRP, Jurina K, Wielaender F, et al. Pregabalin add-on vs dose increase in levetiracetam add-on treatment: a real-life trial in dogs with drug-resistant epilepsy. Front Vet Sci. 2022;9:910038.

Kunda, Shailaja, Reghann G. LaFrance-Corey, Fatemeh Khadjevand, Gregory A. Worrell, and Charles L. Howe (2019), “Systemic evidence of acute seizure-associated elevation in serum neuronal injury biomarker in patients with temporal lobe epilepsy,” Acta Epileptologica, 1 (1).

Lakshmi, Gudimella Sree Ranga, Mohammed Khaliq Mohiuddin, Keloth Mounika, Palkonda Shashikala Reddy, and Devulapalli Krishnaveni (2023), “Genetic Analysis of Cytochrome P450 Polymorphisms in Drug-responsive and Drug-refractory Epileptic patients in Telangana,” The Open Neurology Journal, 17 (1).

Law, Tsz Hong, Holger A. Volk, Yuanlong Pan, Brian M. Zanghi, and Elizabeth J. Want (2018), “Metabolic perturbations associated with the consumption of a ketogenic medium-chain TAG diet in dogs with idiopathic epilepsy,” British Journal Of Nutrition, 120 (5), 484.

Leandro, Kevin, Joana Bicker, Gilberto Alves, Amílcar Falcão, and Ana Fortuna (2019), “ABC transporters in drug-resistant epilepsy: mechanisms of upregulation and therapeutic approaches,” Pharmacological Research, 144, 357.

Li X, Quan P, Si Y, et al. The microRNA-211-5p/P2RX7/ERK/GPX4 axis regulates epilepsy-associated neuronal ferroptosis and oxidative stress. J Neuroinflammation. 2024;21(1):13.

Li Y, Zhou D, Ren Y, et al. Mir223 restrains autophagy and promotes CNS inflammation by targeting ATG16L1. Autophagy. 2019;15(3):478-492.

Li Z, Cao W, Sun H, et al. Potential clinical and biochemical markers for prediction of drug-resistant epilepsy: a literature review. Neurobiol Dis. 2022;174:105872.

Libri, Valentina, Pascal Miesen, Ronald P. van Rij, and Amy H. Buck (2013), “Regulation of microRNA biogenesis and turnover by animals and their viruses,” Cellular and Molecular Life Sciences, Springer Nature.

Liyis, Bryan Gervais de, Sevinna Geshie Tandy, Joana Fourta Endira, Komang Andjani Putri, and Desak Ketut Indrasari Utami (2022), “Anti-high mobility group box protein 1 monoclonal antibody downregulating P-glycoprotein as novel epilepsy therapeutics,” The Egyptian Journal of Neurology Psychiatry and Neurosurgery, Springer Science+Business Media.

Löscher, Wolfgang (2022), “Dogs as a Natural Animal Model of Epilepsy,” Frontiers in Veterinary Science, Frontiers Media.

Löscher, Wolfgang and Graeme J. Sills (2007), “Drug resistance in epilepsy: Why is a simple explanation not enough?,” Epilepsia, Wiley.

Lorenz R, Bernhart SH, Höner Zu Siederdissen C, et al. ViennaRNA Package 2.0. *Algorithms Mol Biol*. 2011;6:26. Published 2011 Nov 24. doi:10.1186/1748-7188-6-26.

Lowe, Helen, Anne E. Keller, Elise Tanzini, Sabrina Aimola, Y. M. Christiana Liu, Maria Zak, Valerie Chan, Jeff Kobayashi, and Elizabeth Donner (2021), “Ketonuria and Seizure Control in the Medium Chain Triglyceride and Classic Ketogenic Diets,” Canadian Journal of Neurological Sciences / Journal Canadien des Sciences Neurologiques, 49 (3), 433.

Ma Y. The challenge of microRNA as a biomarker of epilepsy. Curr Neuropharmacol. 2018;16(1):37-42.

McGrath T, Baskerville R, Rogero M, Castell L. Emerging Evidence for the Widespread Role of Glutamatergic Dysfunction in Neuropsychiatric Diseases. Nutrients. 2022;14(5):917. Published 2022 Feb 22. doi:10.3390/nu14050917.

Mandigers, Paul J. J., Simone E. Meijs, Marta Płonek, Koen M. Santifort, and M. Montserrat Diaz Espineira (2024), “Phenotypic characterization and quality of life of Labradoodles with idiopathic epilepsy and epilepsy of unknown cause,” Frontiers in Veterinary Science, 11.

Mao, Shiqi, Jinhan Wu, Jingkai Yan, Weijun Zhang, and Feng Zhu (2023), “Dysregulation of miR-146a: a causative factor in epilepsy pathogenesis, diagnosis, and prognosis,” Frontiers in Neurology, Frontiers Media.

Martins-Ferreira, Ricardo, Bárbara Leal, João Chaves, Laura Ciudad, Raquel Samões, António Martins da Silva, Paulo Costa, and Esteban Ballestar (2022), “Circulating cell-free DNA methylation mirrors alterations in cerebral patterns in epilepsy,” Clinical Epigenetics, 14 (1).

McGann, Amanda M., Grace Westerkamp, Alisha Chalasani, Cole S. K. Danzer, Emma V. Parkins, Valerine Rajathi, Paul S. Horn, Ernest V. Pedapati, Durgesh Tiwari, Steve C. Danzer, and Christina Groß (2023), “MiR-324-5p inhibition after intrahippocampal kainic acid-induced status epilepticus does not prevent epileptogenesis in mice,” Frontiers in Neurology, 14.

McSweeney, K. Melodi, Ayal B. Gussow, Shelton S. Bradrick, Sarah A. Dugger, Sahar Gelfman, Quanli Wang, Slavé Petrovski, Wayne N. Frankel, Michael J. Boland, and David B. Goldstein (2016), “Inhibition of microRNA 128 promotes excitability of cultured cortical neuronal networks,” Genome Research, 26 (10), 1411.

Majdi A, Mahmoudi J, Sadigh-Eteghad S, et al. The interplay of microRNAs and post-ischemic glutamate excitotoxicity. Neurol Sci. 2016;37(11):1765-1771.

Mahon E, Marsh O, Uriarte A, Stabile F. The effect of oral zonisamide treatment on serum phenobarbital concentrations in epileptic dogs. Front Vet Sci. 2024;11.

Mealey KL, Owens JG, Freeman E. Canine and feline P-glycoprotein deficiency: What we know and where we need to go. J Vet Pharmacol Ther. 2023;46(1):1-16. doi:10.1111/jvp.13102.

Mellios N, Feldman DA, Sheridan SD, et al. MeCP2-regulated miRNAs control early human neurogenesis. Mol Psychiatry. 2018;23(4):1051-1065.

Mesraoua B, Brigo F, Lattanzi S, et al. Drug-resistant epilepsy: definition, pathophysiology, and management. J Neurol Sci. 2023;452:120766.

Milne, Marjorie (2018), "Investigations on the pathophysiology of canine idiopathic epilepsy," Minerva Access (University of Melbourne), The University of Melbourne.

Morita-Sherman M, Trinka E, Kwan P, et al. Precision medicine for epilepsy: challenges and perspectives for an optimized clinical care pathway. Front Neurol. 2025;16:1644835.

Moustafa, Mervat, Noha T. Abokrysha, Nermeen Ahmed El-Desoukey, Dalia Gamil Amin, Nesma Mounir, and Dalia M. Labib (2020), "Role of circulating miR 194-5p, miR 106b, and miR 146a as potential biomarkers for epilepsy: a case-control study," The Egyptian Journal of Neurology Psychiatry and Neurosurgery, 56 (1).

Murgia, Federica, Antonella Muroni, Monica Puligheddu, Lorenzo Polizzi, Luigi Barberini, Gianni Orofino, Paolo Solla, Simone Poddighe, Francesco Del Carratore, Julian L. Griffin, Luigi Atzori, and Francesco Marrosu (2017), "Metabolomics As a Tool for the Characterization of Drug-Resistant Epilepsy," Frontiers in Neurology, 8.

Myrou, Athena, Konstantinos Barmpagiannos, Aliki Ioakimidou, and Christos Savopoulos (2025), "Molecular Biomarkers in Neurological Diseases: Advances in Diagnosis and Prognosis," International Journal of Molecular Sciences, 26 (5), 2231.

Nowakowska, Marta, Muammer Üçal, Marios Charalambous, Sofie Bhatti, Timothy Denison, Sebastian Meller, Gregory A. Worrell, Heidrun Potschka, and Holger A. Volk (2022), "Neurostimulation as a Method of Treatment and a Preventive Measure in Canine Drug-Resistant Epilepsy: Current State and Future Prospects," Frontiers in Veterinary Science, Frontiers Media.

Oberbauer, Anita M. (2003), "The Genetics of Epilepsy in the Belgian Tervuren and Sheepdog," Journal of Heredity, 94 (1), 57.

Olszewska, Agnieszka, Martin J. Schmidt, Klaus Failing, J. Nicpoń, Przemysław Podgórski, and Marcin Wrzosek (2020), "Interictal Single-Voxel Proton Magnetic Resonance Spectroscopy of the Temporal Lobe in Dogs With Idiopathic Epilepsy," Frontiers in Veterinary Science, 7.

Packer, Rowena M. A., Nadia Shihab, Bruno Benetti Junta Torres, and Holger A. Volk (2014), "Clinical Risk Factors Associated with Anti-Epileptic Drug Responsiveness in Canine Epilepsy," PLoS ONE, 9 (8).

Pekcec A, Unkrüer B, Stein V, et al. Over-expression of P-glycoprotein in the canine brain following spontaneous status epilepticus. Epilepsy Res. 2009;83(2-3):144-151.

Peña-Bautista C, Tarazona-Sánchez A, Braza-Boils A, et al. Plasma microRNAs as potential biomarkers in early Alzheimer's disease. Sci Rep. 2022;12:15589.

Peplow PV, Martinez B. MicroRNAs as potential biomarkers in temporal lobe epilepsy and mesial temporal lobe epilepsy. Neural Regen Res. 2022;18(4):716.

Perucca E, French J, Bialer M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol. 2007;6(9):793-804.

Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 2002;30(9):e36.

Phochantachinda, Sataporn, Boonrat Chantong, Onrapak Reamtong, and Duangthip Chatchaisak (2023), "Protein profiling and assessment of amyloid beta levels in plasma in canine refractory epilepsy," Frontiers in Veterinary Science, 10.

Pilla, Rachel, Tsz Hong Law, Yuanlong Pan, Brian M. Zanghi, Qinghong Li, Elizabeth J. Want, Jonathan A. Lidbury, Jöerg M. Steiner, Jan S. Suchodolski, and Holger A. Volk (2020), "The Effects of a Ketogenic Medium-Chain Triglyceride Diet on the Feces in Dogs With Idiopathic Epilepsy," Frontiers in Veterinary Science, 7.

Pitkänen A, Löscher W, Vezzani A, et al. Advances in the development of biomarkers for epilepsy. Lancet Neurol. 2016;15(8):843-856.

Pitkänen A, Lukasiuk K, Dudek FE, Staley KJ. Epileptogenesis. Cold Spring Harb Perspect Med. 2015;5(10):a022822.

Pitkänen A, Ndode-Ekane XE, Lapinlampi N, Puhakka N. Epilepsy biomarkers: toward etiology and pathology specificity. Neurobiol Dis. 2018;123:42-58.

Płonek, Marta, Montse M. Diaz-Espineira, Quirine E. M. Stassen, Koen M. Santifort, Peter A. J. Leegwater, and Paul J. J. Mandigers (2022), "Phenotypic characterization of idiopathic epilepsy and epilepsy of unknown cause in Irish Setters," Frontiers in Veterinary Science, 9.

Potschka H, Fischer A, Löscher W, Volk HA. Pathophysiology of drug-resistant canine epilepsy. Vet J. 2023;296-297:105990.

Prida, Liset Menéndez de la, Richard J. Staba, and Joshua Dian (2015), "Conundrums of High-Frequency Oscillations (80–800 Hz) in the Epileptic Brain," Journal of Clinical Neurophysiology, Lippincott Williams & Wilkins.

Rapacz, Anna (2018), "ADVANCES AND LIMITATIONS IN PHARMACOTHERAPY OF EPILEPSY," Acta Poloniae Pharmaceutica - Drug Research, 75 (5), 1069.

Rawat, Chitra, Samiksha Kukal, Ujjwal R. Dahiya, and Ritushree Kukreti (2019), "Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management," Journal of Neuroinflammation, BioMed Central.

Raoof R, Jimenez-Mateos EM, Bauer S, et al. Cerebrospinal fluid microRNAs are potential biomarkers of temporal lobe epilepsy and status epilepticus. Sci Rep. 2017;7(1):3328.

Raoof R, Bauer S, El-Naggar H, et al. Dual-center, dual-platform microRNA profiling identifies potential plasma biomarkers of adult temporal lobe epilepsy. EBioMedicine. 2018;38:127-138.

Reschke CR, Silva LFA, Norwood BA, et al. Potent anti-seizure effects of locked nucleic acid antagomirs targeting miR-134 in multiple mouse and rat models of epilepsy. Mol Ther Nucleic Acids. 2017;6:45-56.

Risio, Luisa De, Sofie Bhatti, Karen R. Muñana, Jacques Penderis, Veronika M. Stein, Andrea Tipold, Mette Berendt, Robyn Farqhuar, Andrea Fischer, Sam Long, Paul J. J. Mandigers, Kaspar Matiasek, Rowena M. A. Packer, Ákos Pákozdy, Ned Patterson, Simon R. Platt, Michael Podell, Heidrun Potschka,

Martí Pumarola, Clare Rusbridge, and Holger A. Volk (2015), “International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs,” BMC Veterinary Research, 11 (1), 148.

Romeo, María V., Elena López-Martínez, J. Berganza, Felipe Goñi-de-Cerio, and Aitziber L. Cortajarena (2021), “Biomarker sensing platforms based on fluorescent metal nanoclusters,” Nanoscale Advances, Royal Society of Chemistry.

Roopra A, Dingledine R, Hsieh J. Epigenetics and epilepsy. Epilepsia. 2012;53(Suppl 9):2-10.

Roncon P, Soukupovà M, Binaschi A, et al. MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy – comparison with human epileptic samples. Sci Rep. 2015;5:14143.

Rozental AJ, Weisbeck BG, Corsato Alvarenga I, et al. The efficacy and safety of cannabidiol as adjunct treatment for drug-resistant idiopathic epilepsy in dogs: a double-blinded crossover study. J Vet Intern Med. 2023;37(6):2291-2300.

Salem, Talal, Cohen Chetty, and Ochran Chetty (2023), “A Review of the Surgical Procedures for the Treatment of Drug-Resistant Epilepsy and Their Seizure Control Outcomes,” Surgical Science, Scientific Research Publishing.

Santana-Gomez C, Engel J, Staba RJ. Drug-resistant epilepsy and the hypothesis of intrinsic severity: what about the high-frequency oscillations? Epilepsia Open. 2021;7:1-12.

Santifort, Koen M., Elise Bertijn, Sofie Bhatti, Peter A. J. Leegwater, Andrea Fischer, and Paul J. J. Mandigers (2022), “Phenotypic Characterization of Idiopathic Epilepsy in Border Collies,” Frontiers in Veterinary Science, 9.

Sarangdhar, Mayuresh Anant, Sarah Akel, Saman Hosseini Ashtiani, Markus Axelsson, and Johan Zelano (2025), “Association between pro-inflammatory proteins and neurofilament in plasma from persons with epilepsy,” BMC Medicine, 23 (1).

Schmidt, Dieter and Wolfgang Löscher (2005), “Drug Resistance in Epilepsy: Putative Neurobiologic and Clinical Mechanisms,” Epilepsia, 46 (6), 858.

Schmidt, Dieter and Wolfgang Löscher (2009), “New Developments in Antiepileptic Drug Resistance: An Integrative View,” Epiliepsy currents/Epilepsy currents, 9 (2), 47.

Seppälä, Eija H., Tarja S. Jokinen, Masaki Fukata, Yuko Fukata, Matthew T. Webster, Elinor K. Karlsson, Sami Kilpinen, Frank Steffen, Elisabeth Dietschi, Tosso Leeb, Ranja Eklund, Xiaochu Zhao, Jennifer J. Rilstone, Kerstin Lindblad-Toh, Berge A. Minassian, and Hannes Lohi (2011), “LGI2 Truncation Causes a Remitting Focal Epilepsy in Dogs,” PLoS Genetics, 7 (7).

Shah, Sandip, Manan Shah, and Bhavin Parekh (2025), “Epilepsy and its driving forces: understanding the forces behind epileptical pathogenisis,” arXiv (Cornell University).

Shi, Jianwei, Jing Xie, Zesheng Li, Xiaosong He, Penghu Wei, Josemir W. Sander, and Guoguang Zhao (2025), “The Role of Neuroinflammation and Network Anomalies in Drug-Resistant Epilepsy,” Neuroscience Bulletin, 41 (5), 881.

Shlobin, Nathan A. and Josemir W. Sander (2022), “Current Principles in the Management of Drug-Resistant Epilepsy,” CNS Drugs, 36 (6), 555.

Shu P, Fu H, Zhao X, et al. MicroRNA-214 modulates neural progenitor cell differentiation by targeting Quaking during cerebral cortex development. Sci Rep. 2017;7:8014.

Silva, Luiz Fernando Almeida, Cristina R. Reschke, Thanh Ngoc Nguyen, Elena Langa, Amaya Sanz-Rodriguez, Rogério R. Gerbatin, Fernanda R. Temp, Mayara Lütchemeyer de Freitas, Ronán Conroy, Gary P. Brennan, Tobías Engel, and David C. Henshall (2020), “Genetic deletion of microRNA-22 blunts the inflammatory transcriptional response to status epilepticus and exacerbates epilepsy in mice,” Molecular Brain, 13 (1).

Silva-Alves, Mariana S., Rodrigo Secolin, Benilton S. Carvalho, Clarissa Lin Yasuda, Elizabeth Bilevicius, Marina K. M. Alvim, Renato Oliveira dos Santos, Cláudia Vianna Maurer-Morelli, Fernando Cendes, and Íscia Lopes-Cendes (2017), “A Prediction Algorithm for Drug Response in Patients with Mesial Temporal Lobe Epilepsy Based on Clinical and Genetic Information,” PLoS ONE, 12 (1).

Silvestrino, Marco, Mattia Pirolo, Angelica Bianco, Stefano Castellana, Laura Del Sambro, Viviana Domenica Tarallo, Luca Guardabassi, Andrea Zatelli, and Floriana Gernone (2024), “Idiopathic epilepsy in dogs is associated with dysbiotic faecal microbiota,” Research Square (Research Square).

Singh, Rajveer, Kamalesh Chakravarty, Jitupam Baishya, Manoj Goyal, and P Kharbanda (2020), “Management of Refractory Epilepsy,” International Journal of Epilepsy, 6 (1), 15.

Slota JA, Booth SA. MicroRNAs in neuroinflammation: implications in disease pathogenesis, biomarker discovery and therapeutic applications. Noncoding RNA. 2019;5(2):35.

Sumadewi KT, Harkitasari S, Tjandra DC. Biomolecular mechanisms of epileptic seizures and epilepsy: a review. Acta Epileptol. 2023;5(1):28.

Szydłowska K, Bot A, Niźinska K, Olszewski M, Łukasiuk K. Circulating microRNAs from plasma as preclinical biomarkers of epileptogenesis and epilepsy. Sci Rep. 2024;14(1):708.

Sun, Jijun, Weidong Cheng, Lifeng Liu, Shuxin Tao, Zhangyong Xia, Lifeng Qi, and Min Huang (2016), “Identification of serum miRNAs differentially expressed in human epilepsy at seizure onset and post-seizure,” Molecular Medicine Reports, 14 (6), 5318.

Tan CL, Plotkin JL, Venø MT, et al. MicroRNA-128 governs neuronal excitability and motor behavior in mice. Science. 2013;342(6163):1254-1258.

Tang F, Hartz AMS, Bauer B. Drug-resistant epilepsy: multiple hypotheses, few answers. Front Neurol. 2017;8:301.

Thomas, Kristen T., Christina Groß, and Gary J. Bassell (2018), “microRNAs Sculpt Neuronal Communication in a Tight Balance That Is Lost in Neurological Disease,” Frontiers in Molecular Neuroscience, Frontiers Media.

Varatharajah, Yogatheesan, Brent Berry, Jan Cimbálník, Václav Křemen, Jamie J. Van Gompel, Matt Stead, Benjamin H. Brinkmann, Ravishankar K. Iyer, and Gregory A. Worrell (2018), “Integrating artificial intelligence with real-time intracranial EEG monitoring to automate interictal identification of seizure onset zones in focal epilepsy,” Journal of Neural Engineering, 15 (4), 46035.

Van Vliet EA, Puhakka N, Mills JD, et al. Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: focus on circulating microRNAs. Epilepsia. 2017;58(12):2013-2024.

Velu VK, Ramesh R, Srinivasan AR. Circulating microRNAs as biomarkers in health and disease. J Clin Diagn Res. 2012;6(10):1791-1795.

Verdoodt, Fien, S.F.M. Bhatti, Jenifer Molina, Luc Van Ham, Lynn Vanhaecke, Greet Junius, Lieselot Hemeryck, and Myriam Hesta (2025), "Plasma metabolome reveals altered oxidative stress, inflammation, and amino acid metabolism in dogs with idiopathic epilepsy," Epilepsia, 66 (4), 1315.

Verdoodt, Fien, Sofie Bhatti, Karla Kragic, Luc Van Ham, Lynn Vanhaecke, Myriam Hesta, and Lieselot Hemeryck (2024), "Towards a better understanding of idiopathic epilepsy through metabolic fingerprinting of cerebrospinal fluid in dogs," Scientific Reports, 14 (1).

Vezzani, Annamaria, Silvia Balosso, and Teresa Ravizza (2019), "Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy," Nature Reviews Neurology, 15 (8), 459.

Vezzani, Annamaria, Bethan Lang, and Eleonora Aronica (2015), "Immunity and Inflammation in Epilepsy," Cold Spring Harbor Perspectives in Medicine, Cold Spring Harbor Laboratory Press.

Viswambharan V, Thanseem I, Vasu MM, Poovathinal SA, Anitha A. miRNAs as biomarkers of neurodegenerative disorders. Biomark Med. 2017;11(2):151-167.

Wang J, Tan L, Tan L, et al. Circulating microRNAs are promising novel biomarkers for drug-resistant epilepsy. Sci Rep. 2015;5:10201.

Wang J, Yu JT, Tan L, et al. Genome-wide circulating microRNA expression profiling indicates biomarkers for epilepsy. Sci Rep. 2015;5:9522.

Wang Y, Wang Y, Chen Y, et al. Circulating microRNAs from plasma small extracellular vesicles as potential diagnostic biomarkers in pediatric epilepsy and drug-resistant epilepsy. Front Mol Neurosci. 2022;15:823802.

Weissl J, Hülsmeyer V, Brauer C, et al. Disease progression and treatment response of idiopathic epilepsy in Australian Shepherd dogs. J Vet Intern Med. 2012;26(1):116-125. doi:10.1111/j.1939-1676.2011.00853.xWiedrick JT, Phillips JI, Lusardi TA, et al. Validation of microRNA biomarkers for Alzheimer's disease in human cerebrospinal fluid. J Alzheimers Dis. 2019;67(3):875-891.

Wu Z, Liu Y, Huang J, Huang Y, Fan L. MiR-206 inhibits epilepsy and seizure-induced brain injury by targeting CCL2. Cytotechnology. 2019;71(4):1003-1015.

Xie G, Chen H, He C, Hu S, Xiao X, Luo Q. The dysregulation of miRNAs in epilepsy and their regulatory role in inflammation and apoptosis. Funct Integr Genomics. 2023;23:1-15.

Yakimov AM, Timechko EE, Paramonova AI, et al. Hypotheses of development and strategies for overcoming drug resistance in epilepsy. Part I: hypotheses of development. Epilepsy Paroxysmal Conditions. 2025;16(4):375-389.

Yonezawa T, Takenouchi S, Motegi T, et al. Lipid metabolites and nitric oxide production in the cerebrospinal fluid and plasma of dogs with meningoencephalitis of unknown origin and idiopathic epilepsy: a pilot study. Front Vet Sci. 2024;11:1389893.

Yu, Rita, Eun Joo Lee, Joon Soo Lee, Hoon-Chul Kang, and Heung Dong Kim (2022), “A Mixed-Lipid Diet (Medium-Chain and Long-Chain Triglycerides) for Better Tolerability and Efficiency in Pediatric Epilepsy Patients,” Annals of Child Neurology, 30 (4), 164.

Yu, Yoshihiko, Daisuke Hasegawa, Aki Fujiwara-Igarashi, Yüji Hamamoto, Shunta Mizoguchi, Takayuki Kuwabara, and Michio Fujita (2017), “Molecular cloning and characterization of the family of feline leucine-rich glioma-inactivated (LGI) genes, and mutational analysis in familial spontaneous epileptic cats,” BMC Veterinary Research, 13 (1).

Zabrodskaya, Yulia M., Olga N. Gaykova, Mikhail V. Alexandrov, Natalia Paramonova, Darya Sitovskaya, Anastasya V. Litovchenko, T. B. Соколова, S. E. Cherenkova, and E. Д. Бажанова (2022), “Epileptic Focus in Drug-Resistant Epilepsy: Structure, Organization, and Pathophysiology,” in IntechOpen eBooks, IntechOpen.

Supplementary material

miRNA	sequence	F primer	Tm	length
for normalisation:				
cfa-miR-27b	UUCACAGUGGCUAAGUUCUGC	GCTTCACAGTGGCTAAGTTCTGC	58.9	23
cfa-miR-29a_R-1	UAGCACCAUCUGAAAUCGGUUA	GCTAGCACCATCTGAAATCGGTTAG	58.2	25
For control vs naïve epileptics:				
cfa-miR-381	UAUACAAGGGCAAGCUCUCUGU	GCTATACAAGGGCAAGCTCTCTG	57.7	23
cfa-miR-214_R-1	ACAGCAGGCACAGACAGGCAGU	ACAGCAGGCACAGACAGGCA	61.5	20
cfa-miR-224	CAAGUCACUAGUGGUUCCGUUU	GCAAGTCACTAGTGGTTCCGTTT	57.9	23
For responders vs refractory:				
cfa-miR-210_L-1	ACUGUGCGUGUGACAGCGGCUGA	GTGCGTGTGACAGCGGCTGA	63.1	20
		GTGCGTGTGACAGCGGCTG	62	19
cfa-mir-223-p5	UGUCAGUUUGUCAAAUACCCC	CGCTGTCAGTTTGTCAAATACC	55	22
cfa-miR-129_1ss21CT	CUUUUUGCGGUCUGGGCUUGC	CTTTTTGCGGTCTGGGCTTGC	60.1	21

Table S1. Primer sequences used for RT-qPCR identification of microRNA biomarkers

miRNA	Mean Resistant	Mean Responder	Fold Change (Resistant vs Responder)	Expression Level	P Value	ROC_AUC	Cut Off	Sensitivity	Specificity	Targets
cfa-miR-210_L-1	536.0	227.8	2.35	med	2.2E-02	0.99	29.85	1.00	1.00	
cfa-miR-223-p5	436.1	225.9	1.93	med	1.79E-02	0.98	31.69	1.00	1.00	many
cfa-miR-129_1ss21CT	235.0	99.9	2.35	med	3.46E-02	0.96	20.86	1.00	1.00	

Table S2. Differentially expressed circulating miRNAs distinguishing resistant and responder groups

miRNA	Mean Control	Mean Epilepsy	Fold Change (Naïve Epilepsy vs Controls)	Expression Level	P Value	ROC_AUC	Cut Off	Sensitivity	Specificity	Cumulative Sensitivity	Cumulative Specificity	Target
cfa-miR-381	139.2	367.8	2.64	med	3.50E-03	0.9326	83327.89	0.86	0.86	0.86	1.00	
cfa-miR-214_R-1	412.9	856.8	2.08	hi	5.75E-03	0.8982	5333.18	0.71	0.71	1.00	1.00	many
cfa-miR-224	254.0	475.7	1.87	med	8.28E-03	0.8917	462366.7	0.86	0.86	1.00	1.00	many

Table S3. Differentially expressed circulating miRNAs in naïve epilepsy versus controls

Notes

- ROC_AUC: Area under the receiver operating characteristic curve.
- Fold change values >1 indicate upregulation in the comparison group listed.
- “med” = medium expression level; “hi” = high expression level.